ETIOLOGY OF ACUTE GANGRENOUS INFECTIONS OF ANIMALS: A DISCUSSION OF BLACKLEG, BRAXY, MALIGNANT EDEMA AND WHALE SEPTICEMIA

STUDIES ON PATHOGENIC ANAEROBES. I *

HILDA HEMPL HELLER

From the George Williams Hooper Foundation for Medical Research, San Francisco

INTRODUCTION

A piece of work on which I have been engaged, concerning the tissue-invading anaerobes, involved the collection of as many strains of such organisms as possible. At the suggestion of Dr. Karl F. Meyer, a fairly large number of strains of animal origin were collected and analyzed. It was thought that, besides being in itself of economic importance, the investigation of such a collection would be of assistance in the study of organisms causative of gas gangrene in man, for which branch of research the available strains of human origin were found inadequate. As these animal strains in turn threw new light on the subject of the infections of animals, it was thought appropriate to present under the above title certain data in connection with them. It also seemed desirable to make a rather extensive critical review of the literature of anaerobic animal infections.[1]

There are many obscure points concerning the anaerobic invasions of animals. Human wound infections with their resulting cases of gas-gangrene have been studied extensively during the period of the war and they are found to be varied in their etiology and frequently polyspecific in their nature. The anaerobic organisms causative of human gas gangrene are usually placed in what I believe to be three important groups, which are considered by some workers (Weinberg and Séguin (b), the Anaerobe Committee) to be three species, namely, Vibrion septique Pasteur (the bacillus of Ghon and Sachs); B. oedematiens Weinberg and Séguin (closely related to but not identical with B. oedematis maligni II of Novy); and B. welchii (B. phleg-

* This work was commenced during the author's tenure of the Alice Freeman Palmer Fellowship of Wellesley College.

[1] On account of the length of this paper it was found necessary to omit from the Journal a large portion of the literary review which was included in Parts I and III. This portion is included in the author's reprints and in the "Collected Reprints of the Hooper Foundation," volume 5, 1919-1920.

mones emphysematoseae Fraenkel, B. perfringens Veillon and Zuber). Rarely other invading organisms are found which are not to be identified with the above. I cite B. histolyticus, B. fallax and B. aerofoetidus Weinberg and Séguin, B. egens Stoddard, and have myself found a few such organisms that appear to be new. In the war literature there seem to be a host of faulty identifications, of associations of unrelated organisms, and of descriptions of mixed cultures. I shall confine myself in this paper to a discussion of the infections of animals.[2]

I. A General Discussion of the Principal Organisms Involved in the Anaerobic Infections of Domestic Animals

The differentiation of the disease called blackleg from that which is usually called malignant edema has been undertaken in all modern textbooks of veterinary pathology. The criteria used for such differentiation are usually seriously open to criticism, and in some cases are the cause of decades of misunderstanding of the subject. So serious have these misconceptions become that vibrion septique strains have been used as standards by which to identify B. chauvoei. I cannot otherwise explain the statements of M. Nicolle, Cesari, and Raphael, who correlate the two organisms. The fact that the Pasteur Institute sent this laboratory a strain of the vibrion septique group, which was known as one of LeClainche and Vallée's classic blackleg strains, lends color to the suggestion that Nicolle, Cesari, and Raphael did not work with true strains of B. chauvoei. Weinberg and Séguin come to the conclusion, through a review of the literature, that vibrion septique and B. chauvoei are identical (b). I find it impossible to confound the organisms of these two types if they are in pure culture and if adequate facilities are at hand for their study.

It may be broadly stated that the anaerobic invaders of mammalian tissue form a large group, the members of which are not truly parasitic

[2] The bibliography of these diseases is sometimes difficult to quote in an orthodox fashion. Various authors of textbooks give long lists of articles consulted by them, and frequently in the account which follows such a bibliography no indication is given as to which of the many authors is responsible for the statements made in the text. A large number of references in early volumes of veterinary journals are difficult of access. One textbook gives an excellent account of braxy with no bibliography at all, while other important textbooks give references to a few but not to all of their authorities. In general, I have tried, in referring to an authority who quotes from another, to give the quoted statements verbatim; for the sake of clearness I have inserted the page number with the name of the author in the text. A complete alphabetical list of references will be found at the end of the paper. The letter "p" before a number refers to page numbers in this paper.

but may become so under diverse conditions. The species of the invaders are many, and they may be organized into groups which may best be likened to genera. Chief among these groups in incidence in animal infections is the vibrion septique group, which has a wide range of pathogenicity among animals. Second in incidence is the blackleg group, which has a narrower range of pathogenicity among domestic animals. Oedematiens group and Welch group infections may occur in animals, but they are comparatively rare. One should keep in mind the possibility of the occurrence of infection by organisms of other groups, and the question of invasion initiated by proteolytic organisms is to my mind entirely open. We are only on the threshold of the study of the anaerobic tissue invaders. Careful investigation of a large number of strains is necessary before definite generalizations concerning them are warranted. As far as my investigations go, I am prepared to define with some accuracy the principal groups of anaerobic invaders. Later researches may compel me to change somewhat the details of these definitions. Such definitions are here given for the vibrion septique and blackleg groups only.

Recently a bacteriologist declared to me in a private communication that a certain strain corresponded to the descriptions of the bacillus of blackleg which were "found in the literature." The culture proved to contain several anaerobic organisms of various types. On thinking the matter over in the light of the reading that I had been doing I could not blame him for his diagnosis. Perhaps this paper will serve to stimulate a more exact study of a sadly misunderstood group of bacteria. It should be said, and with emphasis, that the careful study of the organisms involved is of infinitely more value than any unstandardized pathologic observations or any epidemiologic ones. In many cases in which reports are given concerning blackleg infections or malignant edema infections it is impossible to tell which type of organism was present, whether both types were present, or whether an organism of another group may not have been present. No one is qualified to make a statement as to the etiologic factor in an anaerobic infection without bacteriologic investigation, and the literature contains so many confusing statements as to the bacterial entities involved that no one but the fortunate bacteriologist who has several strains to study and the time to study them is qualified to determine even the group affinities with certainty. When, however, the nature of the

groups is understood, and when a technic is developed for their differentiation, one does not understand how the two could ever have been confused. They stand clear and separate as night and day, and mixtures of the two are quickly recognized.

1. CRITERIA OF DIFFERENTIATION EVOLVED DURING THE PRESENT RESEARCH

To save much confusion I shall here insert a definite statement as to the behavior in my hands of the two chief types of anaerobic animal invaders. I do this with due apology to others[3] who have adequately described these types, in order that the reader may understand definitely what my criteria of distinction are. I base my group identifications of the vibrion septique type on two strains of this type (VS and MP) sent to Miss Robertson and to Dr. Meyer by the Pasteur Institute. They are not specifically identical but are both referable to the group as I conceive it. Both are supposed to have been strains of Pasteur's. The blackleg group identification with B. chauvoei is based on the description of Arloing, Cornevin, and Thomas, see reprint.

(a) The Blackleg Group.—I should define the blackleg group[4] as follows:

1. Pathogenic Characters: Organisms producing a deeply hemorrhagic inflammation and necrosis in the muscles of the guinea-pig, with some sanguinolent infiltration, with little or no gas production, little interstitial regional edema, and only local injection of the peritoneum and rarely any injection of the small intestines. In guinea-pigs, dying of blackleg infection, bile frequently enters the small intestines in large quantities; the gallbladder is usually empty, the intestines do not contain an unusual amount of gas; the peritoneal surfaces are not excessively moist. The blackleg organisms are not highly pathogenic for laboratory animals when compared with the organisms of the vibrion septique group; some strains are in my hands pathogenic for rabbits.

2. Morphologic and Staining Characters: Bacilli that are usually gram-negative (carbol methyl violet Gram stain, see p. 401, b). In the vegetative form, in animal tissue and in meat medium, they usually stain uniformly and palely. In the orgont[6] form and in the sporulating form they may or may not stain clearly, depending on the amount of carbohydrate in the

[3] Von Hibler (a and c) has a clear understanding of both groups. Markoff, Wulff, and several other workers have a definite understanding of blackleg. Ghon and Sachs, Weinberg and Seguin (b), and Robertson (a) have adequately described the vibrion septique type. Meyer (a and c) and McIntosh give the salient characters of both types, and lastly both types are described by the Medical Research Committee (Special Report No. 39).

[4] A detailed classification of the species that are represented in my collection and make up these groups, as well as certain related groups, is in process of elaboration.

medium. In case they stain clearly they stain less intensely than do vegetative forms; in case the protoplasm is granular, the granules may stain strongly, taking the Gram stain; the remaining substrate is pale and usually gram-negative. The vegetative forms are comparatively slender, usually averaging in a 24-hour meat culture at least four times as long as wide. Actual measurements vary greatly. The vegetative and sporulating forms of most strains show little tendency to form filaments. I have never seen strains of this group which form filaments on the liver of the guinea-pig. The orgont forms are often far larger than the vegetative forms. They are extremely polymorphic, varying greatly within the group. Some are rods, some are rounded or pointed oval forms, some are lemon shaped, some are almost spherical. Spores form early in the development of a culture—in meat medium after approximately 20 hours' growth, and sporulating rods may usually be found at the site of inoculation in the guinea-pig. Spores are always oval, never spherical; they may vary greatly in their proportions and size in one strain, sometimes being long with parallel sides; they are usually situated subterminally in the bacillus (though terminal and median spores occur) and they frequently lie sidewise in the bacillus. This character is more usual for some strains than for others; it is not characteristic of blackleg alone. Rods containing two spores are occasionally seen.

3. Cultural Characters: Strictly anaerobic organisms generally of shy growth habit, which show no proteolytic action on meat, serum, or egg mediums, but which liquefy gelatin. They do not in my hands grow on fresh brom-cresol milk alone but grow well even in old milk with blood in it, forming in 2-4 days a soft clot which is torn by ascending gas and is usually fragmentable by shaking. They split glucose readily, producing gas in meat medium and turning it pink. They are strictly nonproteolytic in the anaerobist's sense of the term. They are violently hemolytic; they autoagglutinate with extreme readiness; they are flagellate and motile (see p. 401, a).

Their deep colonies in liver agar are small, rather slow to develop, fundamentally lenticular in structure and are subject to variations according to species. The colonies rarely show tufted projections and do so only after two or three days' incubation. These projections are not loosely woolly but are clumpy and downy.

(b) The Vibrion Septique Group.—I should define the Vibrion septique group as follows:

1. Pathogenic Characters: Organisms producing a moderately marked serohemorrhagic inflammation of the muscles of the guinea-pig, marked interstitial infiltration, a moderate degree of serous subfacial regional edema and usually a large amount of gas; and producing almost invariably extensive

[5] Orgont. participial stem of ὀργάω 'swell', properly 'swell and teem with moisture', used, for example, of fruit swelling as it ripens. Compare schizont from σχίζω 'split.' I would suggest orgont as an adjective descriptive of the organisms that have lost their bacillary form or size and have swollen, presumably preparatory to sporulation. "Orgont form" may be taken to include all swollen forms in which spores are not visible, whether the fate of such an individual is sporulation or dissolution or even division. "Orgont" may be used as a noun, like schizont. Rods which do not swell preparatory to sporulation may be said to sporulate without "orgont formation." Orgonts may or may not show protoplasmic disorganization.

injection of the serous membranes and of the intestines. In guinea-pigs dying of a vibrion septique infection the bile is freely discharged into the small intestines in large quantity, the gallbladder is usually empty, and the intestines contain a large amount of gas. The peritoneal surfaces are excessively moist.

2. Morphologic and Staining Characters: Organisms usually gram-positive (carbol methyl violet Gram stain, see p. 401, b), though not intensely so, as are the organisms of Welch and Bifermentans type. In the vegetative form in animals and in meat medium they stain uniformly and rather deeply with anilin dyes. In the orgont form, and frequently in the sporulating form, they stain irregularly. In the animal body a localization of deeply staining protoplasm in one or both ends is frequent, and barred rods are common in certain strains. Granules of protoplasm are gram-positive and take stains deeply; the substrate may be very pale and may even lose a Gram stain. The vegetative forms are usually comparatively chunky in meat medium and also in the muscles of experimental animals. When growing in meat medium, forms are usual in the case of most strains, which on the average are not more than three times as long as thick. Filaments are occasional in animal muscle and in meat medium, they are usual in the serous cavities of animals and reach their greatest size on the surface of the liver of animals. Some strains do not form such filaments until some time after the death of the animal. The orgont forms are extremely polymorphic in shape in animal tissue and are sometimes so in serum mediums, and they are not abundant in meat medium, sporulation occurring in such medium without the formation of large orgonts. "Chunky oval forms" are the common orgonts found in animal tissue. In general the orgont forms are smaller than the corresponding forms in the blackleg group. Spores form early in the development of a culture—in meat medium after, roughly, 18 hours' incubation; they may or may not be present at the time of the death of the animal. Spores are oval, never spherical and never square ended. They vary somewhat in size but usually very little in the ratio of their width to their length. They may be terminal or median in their location in the bacillus; median spores are frequent. Spores are usually symmetrically placed in the rod; two strains (AS and BRH) in my possession form at times spores which are placed distinctly sidewise. The formation of two spores in one rod is exceedingly rare.

3. Cultural Characters: Strictly anaerobic organisms of comparatively energetic growth habit, which show no proteolytic action on meat, serum, or egg mediums, but liquefy gelatin. They produce, in milk, in 1-4 days, gas and a soft clot which, at an early stage, is fragmentable by shaking but later stiffens, and is torn by small bubbles of ascending gas. They split glucose readily producing gas in 18 hours in meat medium and turning it pink. They are not so violently hemolytic as members of the blackleg group. They occasionally auto-agglutinate in liver broth. They are motile in actively growing neutral or alkaline cultures. They reach the zenith of their growth in about 20 hours in most mediums, and after three days no longer proliferate.

Their deep colonies in liver agar are large, quick to develop, and woolly, showing rarely (strains WS and VS), and only early in liver peptone agar, evidences of a lenticular origin; they are subject to variation according to species.

2. DIFFERENTIATION OF THE ORGANISMS INVOLVED IN ANIMAL INFECTION: JUDGED CHIEFLY BY THEIR ACTION IN THE ANIMAL BODY

The discussion of the criteria which have been considered as of value in the determination of these groups brings us to the history of the subject.

(*a*) *Differentiation on the Grounds of Putrefactive Action.*—The early history of the vibrion septique and malignant-edema entities is one of the most interesting in bacteriology. Most authors find the descriptions of Pasteur and of Koch too fragmentary to be significant. This I believe to be a mistake. Though it is quite impossible to make any specific determinations on the grounds of the descriptions given by Pasteur and by Koch, it is quite possible to place their organisms approximately. We are, however, forced to pin our sources down to two classic and oft quoted descriptions.

Pasteur's description is significant. He inoculated into a guinea-pig material from a cow that had been dead for three days, presumably of anthrax, and into another, material from a horse that had been dead for one day, presumably of the same disease. We are told only that the disease was suspected and that Pasteur saw anthrax bacilli in the smears. The two guinea-pigs died, showing *désordres epouvantables;* the muscles of the abdomen and of the four legs were extremely "inflamed." Gas was present in the form of pockets, especially in the axillae (*"Ca et là, particulièrement aux aisselles, des poches de gaz"*). The liver and lungs were pale, the spleen normal but at times diffluent and the organisms were present in great numbers in the serosity of the abdomen and about the intestine. They were filamentous in the serous cavities, and regularly became septicemic in the guinea-pig before death. I interpret Pasteur's description to be that of an infection caused by an organism of the vibrion septique group as described on page 389, b. To be emphasized are: extreme inflammation of the muscles, gas production, formation of filaments, infestation of the abdominal serous membranes, septicemia. We are in no position to decide whether either of the two guinea-pigs that were infected from the cow and the horse, and such animals as were infected from them, died of a mixed infection or of a pure one. But surely the infection described by Pasteur was not of the type that shows great contamination by proteolytic organisms, because in such infections gas is not confined in bubbles in the axillae, but forms a large bubble in the destroyed tissue. One is forced to read into such a description as this the things that are not mentioned, as well as those that are. A proteolytic infection in which proteolytic organisms are at all abundant has such an odor of putrefaction that the abductor does not omit to state the presence of such an odor. That other animals which Pasteur inoculated with earth did have a mixed proteolytic infection is of course more than probable. Such infections he later refers to several times: "Un animal va mourir de la putridité septique qui nous occupe, car cette devrait être définie la putréfaction sur le vivant." And: "La septicémie ou putréfaction sur le vivant est-elle une maladie unique? Non; autant de vibrions, autant de septicémies diverses, benignes ou terribles . . ." which shows that Pasteur never pretended that one type of organism was responsible for these infections. This apparently contradicts Perret's statement that Pasteur identified his vibrion septique with the septicemia of Davaine which produced no macroscopic lesions.

It may be concluded, I believe, that Pasteur sometimes worked with mixed inoculum, wherefore he mentioned putrid infections, but that he at other times purified his inoculum by animal passage and that it is from such strains, of which two are included in my collection, that we now have the type of organism that we today recognize as the vibrion septique of Pasteur, which is a strictly nonproteolytic organism. The fact that the *vibrions septiques* of Pasteur, of which we have strains, cause lesions directly comparable with those described by Pasteur on page 793 makes this a type more definite. I do not mean to imply specific determination on the grounds of these old investigations, as I believe specific determinations to be at all times questionable when made from printed descriptions.

The Malignant Edema of Koch: To read the original article in which Koch proposes the name "malignes Oedem" (Zur Aetiologie des Milzbrandes) after spending months in the consideration of the work of his followers who have discussed the subject of "malignant edema" and its causative organisms: to read it in the light of familiar acquaintance with the organisms common in wound infection—is a most illuminating experience. Koch did better work on the subject of anaerobic infection in 1881, with no culture mediums that would grow his organisms, with no Gram stain, with little to guide him, than did any one else till Novy described his B. oedematis maligni II in 1894. The abyss of bacteriologic misunderstanding into which some of Koch's countrymen have today fallen (W. Kolle, Ritz, and Schlossberger, 1918) could all have been avoided by the perusal of Koch's article of 1881, and by the judicious use of laboratory animals. It lies in the nature of things that the malignant edema organisms encountered by Koch were not all the same. But the type described by him on pages 52-56 of "Zur Aetiologie des Milzbrandes" is a type that we know today, and it is not the type to which the vibrion septique of Pasteur belongs. The photomicrographs (table 8, figs. 43, 44, 45) are, however, probably taken from organisms which do belong to the group of the vibrion septique of Pasteur.

At the moment of the writing of his description of the lesions produced by him in experimental animals and termed "malignes Oedem," Koch must have been unfamiliar with the type of lesions described by Pasteur and referred to above. This I judge to be the case because Koch accuses Pasteur of having worked with mixed infections and gives a translation of Pasteur's necropsy findings on the guinea-pigs inoculated from the horse and the cow, allowing himself the liberty of translating "poches de gaz" as "von stinkendem Gas" (noted also by Ghon and Sachs [b, 666]), for which performance I cannot see any justification whatsoever. Koch describes mixed infections following the inoculation of dirt into experimental animals, with their gas and their thin pus-like fluid (Jauche), and he speaks of the peculiar dirty-red coloration of the muscles, which he, quite without justification, interprets to be the color that Pasteur described as due to "inflammation." This type of infection Koch states to be due to a highly complicated mixture of organisms. If one should take this mixture, says he, and inoculate it in any considerable bulk into a new animal, a similar infection will result. But if one should inoculate into a new animal a minute dose from the first animal, one will produce a slower infection, which is caused by a single species of organism. For this type of infection Koch proposed the name "malignes Oedem." It is, I believe, to this description that we must turn for our type of the malignant edema bacillus of Koch, and not to the photomicrographs mentioned above. The infection described is classic. Strange that it should have lain so long forgotten, while the name "malignes Oedem," and the later name B. oedematis maligni Koch, should

have been battered to and fro from laboratory to journal, from journal to textbook, until all semblance of the original pathologic type and of the original organism was lost. Koch's description is as follows: The liquid which fills the subcutaneous connective tissue near the site of inoculation of the experimental animal (sp. ? probably the guinea-pig [6]) is not purulent, but is a pale reddish serum without odor and without gas formation. It contains only one kind of bacilli which in size and shape are almost exactly like anthrax bacilli. (They are a little slenderer and are not grouped in chains like anthrax bacilli [55]). Usually they are nonmotile and only occasionally does one see a movement on the part of an isolated rod. The internal organs of the animal show few changes. The spleen is usually enlarged and darkened, the lung is pale and grayish red. The presence of organisms in the blood varies. The bacilli are short and do not form chains until some time after the death of the animal. They are present in numbers on the serous lining of the abdomen even when no organisms are found in the blood. In mice the organisms regularly become septicemic, in other animals they do not always do so. There are also certain points that must be read into this description. No mention is made of changes in the muscle. If a true pure vibrion septique infection had been seen by Koch at the date of the writing of his description, he would most certainly have mentioned the muscle affection produced by that type of organism, which was described by Pasteur as "inflammation," and he would never have dared attack Pasteur so energetically as he did. Koch does not speak of the consistency of the edema present, he simply calls the disease seen by him "malignes Oedem." Neither does he mention injection of the serous membranes of the abdominal cavity; he insists that the internal organs show few changes; he describes no "désordres épouvantables." The description is so clearly given that one does not hesitate to assign Koch's organism to the same group [7] as B. oedematiens Weinberg and Séguin and B. oedematis maligni II. Novy. The oedematiens strains 139 and Domange of Weinberg and Séguin and strain AB of my own isolation (human gas gangrene) all produce in the guinea-pig lesions exactly like those described by Koch (I have not noted spleen changes); the organisms in my hands sometimes become septicemic and sometimes do not, while organisms of the vibrion septique group always become septicemic, at least in the guinea-pig (this character is, however, not a good one as it is based in my case on cultural determination and in Koch's case on microscopic observation); the organisms enumerated above do not form chains on the abdominal serous membranes of the guinea-pig; they are practically nonmotile, while vibrion septique strains are usually motile; they are large, and resemble somewhat roughly B. anthracis, which organisms of the vibrion septique type cannot well be said to do; most important of all, they do not produce marked muscle lesions or changes in the internal organs as do vibrion septique strains almost without exception; and they produce no gas in the connective tissue of the guinea-pig. I hold most positively that the malignant edema bacillus as described by Koch is nonproteolytic, because Koch states that the liquid near the site of inoculation is "ohne Gestank." This opinion I base on my own necropsy findings. Von Hibler's bacillus X in no way resembles Koch's organism; neither does the organism

[6] As the guinea-pig is mentioned several times on subsequent pages in Koch's paper, and as Koch declares that this animal is extremely sensitive to malignant edema, it is probably safe to conclude that Koch saw the disease in that animal. Whether he saw it in rabbits we do not know, but rabbits are not mentioned in the neighboring text.

[7] By "group" I do not here mean organisms that would necessarily be placed in the same genus by the systematist, but organisms producing the same type of lesions in the animal body.

of Ghon and Sachs resemble it. Koch, so often stated to have identified his organism with the vibrion septique of Pasteur, merely says: "die Oedembacillen von ihm (Pasteur) vibrions septiques gennant." As we probably have today no strain called "malignant oedema of Koch," which produced the anatomic picture so well described by Koch, we are not in a position to identify Koch's bacillus specifically with any other organism. We have certainly no right to place it in the vibrion septique group until we have unquestioned strains of the vibrion septique group which produce the lesions described in Koch's classic delineation. But I think we are in a position to place it in the same general group to which B. oedematiens and B. novyi belong. It must, however, be borne in mind that the term "malignes Oedem" was applied to a disease and not to an organism, that Koch and Gaffky used this name for most of their infections that were produced in animals by the inoculation of dirt, and that it is more than probable that among such infections the vibrion septique type was the most usual one. It is unreasonable for us to expect that Koch should differentiate these types. Let us remember when he worked, and under what handicaps. Let us not conclude that Pasteur differentiated his infections with any certainty. Let us remember his confusion of vibrion septique infections with rabies, and remember how few of the followers of Pasteur and of Koch have seen the light in the matter of differentiation of anaerobic infections.

We have here one of those peculiar anachronisms that come about with the lapse of time. Pasteur surely was not clear in his mind as to pure infections and impure infections. He called his "vibrions septiques" putrefactive, but described a nonputrefactive type of infection, and we cannot decide just where his bases of criticism lay. But the strains which have come down to us under the name vibrion septique Pasteur were evidently pure ones and were kept pure in the Pasteur Institute, and we have them today as pure nonproteolytic cultures. Koch, on the other hand, was to my mind perfectly clear as to the difference between putrefactive and nonputrefactive infections; later, he probably isolated his organisms, cultivated them, and gave them to the world. Gaffky also had, for his time, a remarkably clear understanding of putrefactive mixtures and pure infections. To what extent the cultures which were given out were the type described by Koch or the type described by Pasteur, and whether they were of Gaffky's isolation, we cannot tell. The disease described by Gaffky is different from that described by Koch. On page 87 he describes an infection in the guinea-pig entirely similar to that described by Pasteur and he speaks of his organisms as "vibrion septiques." Perhaps it was from one of these guinea-pigs of Gaffky's that the material came from which Koch's photomicrographs were taken. Gaffky also describes another disease of the general type with which we are dealing which was pathogenic for rabbits and mice, and not pathogenic for guinea-pigs. In discussing Koch's organism we are forced to leave Gaffky's organisms out of consideration: it will cause confusion enough to discuss two strains isolated by the same man, let alone those isolated by two men. We must consider that in whatever way we look at these descriptions of Koch's and of Gaffky's, there is an inconsistency between them. These "authentic" cultures of Koch's, if they were pure when given to bacteriologists at large, must quickly have become contaminated, as anaerobe cultures nearly always do in the hands of the aerobist "layman," but they kept their name, "malignes Oedem." And other workers "isolated" other organisms with similar putrefactive contaminations, which produced lesions similar to those produced by the now contaminated cultures of Koch, probably similar infections to those scornfully described by Koch as

mixed. Later von Hibler may have found such cultures, at least he described carefully a proteolytic organism as B. oedematis maligni Koch (von Hibler's X) without the slightest scientific reason for so doing. Weinberg and Séguin (b, 23), and Fraenkel and Zeissler emphasize this point.

Many proteolytic organisms are capable of invading guinea-pig tissue in pure culture when administered in doses of 3 c c or more. This was the basis of Colin's protests against the technic of Pasteur in 1881. I do not wish to be understood as including such dosage in pathogenicity tests. One c c of a 24-hour meat culture is a large dose for a guinea-pig, and I do not employ larger ones.

Ghon and Sachs described an organism from a human case of gas gangrene which they identified culturally with vibrion septique Pasteur. They were unable culturally to compare their organism with the malignant edema bacillus of Koch, as they possessed no culture of Koch's organism. They then patriotically proceeded to call their organism B. oedematis maligni Koch, although the name vibrion septique Pasteur had preference. They did this on the ground of identifications made by German and French workers in the eighties. As Weinberg and Séguin suggest (b, 82), if vibrion septique did not seem appropriate as a name for their organism, surely B. oedematis maligni was no more appropriate, and a new name should have been chosen. The need for such a name was felt, for the organism of Ghon and Sachs was culturally different from the usual conception of B. oedematis maligni, and in pathogenicity it was somewhat different from the usual conception of vibrion septique Pasteur, neither of which organisms had been adequately described, so that later writers (Von Hibler, Meyer, Kitt, Köves, Schlemmer) who have identified organisms with the bacillus of Ghon and Sachs have called it by the name of the describers. These identifications have been numerous because the description given by Ghon and Sachs is detailed and elaborate. Such organisms have usually been defined as nonproteolytic, as forming chains on the liver of animals, and sometimes as nonpathogenic for rabbits. It is, in fact, in recent days, rare to find a record of an infection attributed to the bacillus of malignant edema of Koch, as described by von Hibler, which fact may be interpreted as due to the more careful cultural methods of recent workers.

Von Hibler (a, 88) is emphatic in his statement that the bacillus of Rauschbrand is nonproteolytic. He grouped all organisms found by him which formed woolly colonies, which grew in filaments on the liver of guinea-pigs, and which were nonproteolytic in their action on mediums, under the name "bacillus of Ghon and Sachs." That these strains of von Hibler's were not all of the same species, I am convinced. For example, strain 6 (pl. II, fig. 4) forms on the peritoneal wall of the guinea-pig, 4 hours after death, oval club-shaped forms. Strain 2, which I received in pure culture through the courtesy of Professor W. Frei of Zürich, does not form such club-shaped rods on the peritoneal wall of the guinea-pig, though I have seen 2 other strains of the vibrion septique group that do so in mice. Strain 5 (Kolle u. Wassermann IV. pl. XIX.1) does not form such clubs on the liver of the guinea-pig 6 hours after death. I am emphatically of the opinion that this type of character is specific. Von Hibler grouped strains that formed woolly colonies and that grew in filaments on the liver of animals but which were proteolytic in their action on mediums, under the name B. oedematis maligni Koch. Weinberg and Séguin (b, 24) seriously criticise von Hibler for this classification, saying that his identifications are incomplete, adding that von Hibler never isolated a strain from man that corresponded to this conception, all his human cases of "oedème malign" being attributable to the bacillus of Ghon and Sachs.

Von Hibler isolated his strains of B. oedematis maligni from a mule, from garden earth that was inoculated into an animal, and from a cow that died of puerperal septicemia. Of his cases that were attributable to the bacillus of Ghon and Sachs, 5 were from man and 2 were from cattle. I do not understand why Weinberg and Séguin insist that an organism of this type should come from a human case in order to be genuine. I have, however, in a series of some 200 samples of pathologic material from animals and man, never encountered any strain which corresponds to the bacillus of malignant edema as described by von Hibler. That the strains studied by von Hibler were occasionally impure is witnessed in mute testimonial by two photomicrographs (a, pl. XI, figs. 2 and 16) which unquestionably portray mixed cultures.

Kitt (e, 287) classes as one species most of the anaerobic organisms that infect wounds, and he gives to this conception the name used for the organism of Koch, in its Latinized form, B. oedematis maligni. Of the agar cultures of this organism he states: "Das Gas ist geruchslos. (Von einigen Autoren wird angegeben, das stinkende Gase sich entwickelen; Mischkultur? Verwechslung mit anderen Kadaverbacillen?") He mentions here no proteolytic invaders. But in discussing Rauschbrand (e, 301) he declares that milk may be used to differentiate Rauschbrand from malignant edema, as the latter liquefies it and produces a foul odor (Smith, E. von Hibler). He describes a mode of obtaining B. oedematis maligni by inoculation of earth into mice or guinea-pigs, and holds that if such an infection is a mixed one, another inoculation will give a pure culture of the malignant edema organism. My experience with such infections has been very different from Kitt's. Earth contains many species of anaerobes that will infect mice and guinea-pigs, and mixed infections may occasionally persist as such after several passages, though if a true organism of the vibrion septique group is present, it usually outruns all others in the first or second passage. Von Hibler describes several species of invasive anaerobes that were isolated in this manner. Kitt mentions the fact that spontaneous malignant edema infections are often mixed, but he does not mention any anaerobés as being contaminating organisms.

Hutyra and Marek (33) say of B. oedematis maligni: "Milk is changed in a few days into a watery fluid with gas formation and the collection of a layer of fat on the surface (Smith)"; and they say (41) concerning the blackleg bacillus: "Milk is curdled only imperfectly."

Lehmann and Neumann (500) state that a putrid odor is indicative of malignant edema and absence of such odor is to be taken as indicative of Rauschbrand.

Bongert (198) declares the bacillus of malignant edema produces "höchst übelriechende Gase verschiedener Art."

Grassberger and Schattenfroh (a and b) declare the Rauschbrand bacillus is an exquisite butyric acid bacillus (nonproteolytic), and the malignant-edema bacillus (vibrion septique) is a proteolytic organism. (See, however, reprint.)

Von Werdt (a and b) recognizes the existence of a "true bacillus of malignant edema of Koch" which is proteolytic and to the discussion of which he devotes a chapter: "Malignes Oedem." He also recognizes the existence of a group of organisms which he calls the organisms causative of "Gasbrand" and to which he devotes another chapter: "Gasbrand und seine Erreger." These organisms comprise: Welch-Fraenkel's bacillus, Ghon-Sach's bacillus I., Novy's bacillus, von Hibler's VI, VII, and XI, Ghon-Sach's bacillus II, Klein's B. enteritidis sporogenes, Klein's B. cadaveris sporogenes, Markoff's pseudo-rauschbrand organisms, the bacillus of malignant emphysema of Stolz, the

bradsot bacillus, and the whale septicemia bacillus. These organisms are extremely varied in their affinities and in their pathologic action; some are proteolytic and some are nonproteolytic. Von Werdt's work is in the nature of a compilation only, and in no way tends to clarify the confusion existing in the anaerobic field. It reflects exactly the history and literature of the subject, and may be consulted for valuable details and for its excellent bibliography. It it not critical.

Foth (b, 252) conceives Rauschbrand to be nonproteolytic and the chain-forming organisms to be proteolytic. His chain-forming strains vary in proteolytic action, but he has never found a chain-forming strain which in its behavior approaches the nonproteolytic character of the bacillus of Ghon and Sachs. Foth uses the term "verbandbildende Anaeroben" to include various species.

Wulff, who worked in Foth's laboratory, has a conception similar to that of Foth and calls all proteolytic infections "malignes Oedem."

Markoff divides his strains, as does von Hibler, into blackleg, nonproteolytic chain formers, and proteolytic chain formers (malignes Oedem).

Weinberg and Séguin (93) declare vibrion septique to be nonproteolytic. Robertson (a) emphasizes the same point. Neither reports any such organisms as the malignant edema of Koch-von Hibler.

Eugen Fraenkel and Zeissler, who carefully isolate their strains, have never found such an organism as the malignant edema bacillus of Koch as described by von Hibler.

In the light of my own experience with anaerobes, I must state that until I find in my hands an indubitably pure culture of a highly proteolytic organism, which alone forms filaments on the liver of a guinea-pig and which has more than a history of pathogenicity, I must continue to doubt the identity, nay, the very existence, of B. oedematis maligni Koch, as described by von Hibler. No one would be more interested or more delighted to find and study such a strain than I. If the organism does exist, I think I am safe in stating that many identifications of it have been faulty, and were based on the investigation of impure cultures. All through the anaerobic literature we find references to pathogenic proteolytic organisms and some of these no doubt exist as entities, capable of penetration in pure culture. Such organisms are, however, exceedingly rare. I have found only two such strains in a series of a hundred tissue invading anaerobes, and these two are only slightly proteolytic. I wish at this point to express my thanks to Miss Muriel Robertson, under whom my anaerobic studies began, for the principles regarding anaerobic investigation which she taught me. It was from her that I learned to discredit the purity of a proteolytic pathogenic culture till I had investigated both the purity and the pathogenicity of such a culture with all the resources at my command. Practically all strains that have come into this laboratory with a history of pathogenesis and that have shown a proteolytic action on meat proved either to be no longer pathogenic for guinea-pigs or to contain a primary invader in the form of a nonproteolytic organism.

I have found no proteolytic invaders in cattle, sheep, or hog material.

I wish also to state at this point that in my hands the characters of anaerobes change no more frequently than do those of other organisms. When a culture radically changes its habit it will be found to contain more than one species of organism.

We may sum up the situation by stating that because of the universal occurrence of organisms of sporogenes affinities and because of their frequent contamination of pathogenic cultures, and because of their occasional invasion of living tissue in company with nonproteolytic invaders, "malignant edema" of animals and man has frequently been described as due to a proteolytic organism.

Friedberger and Fröhner (178) and authors of other textbooks emphasize the universal occurrence of the malignant edema organism, apparently confusing it with the ubiquitous proteolytic forms. They say: (189) "Mit dem Fortschritte der Faulniss verlieren die Oedembacillen ihre Wirksamkeit. Nach etwas 2 Monaten hört sie ganz auf. Trocknet man die Bacillen jedoch bei 15—38 c., ehe Faulniss aufgetreten ist, so bewahren sie ihre virulenz dauernd." Of the fact that proteolytic organisms so overgrow pathogens as to render them no longer pathogenic or to eliminate them entirely, I could give a hundred examples from my own findings. Weinberg has shown that the filtrate of B. sporogenes neutralizes the toxin of B. oedematiens in vitro and neutralizes to some extent even the toxin of vibrion septique, but that it does not neutralize the toxin of B. perfringens (B. welchii). Certain workers (Donaldson and Joyce, Mayo-Robson) have even used cultures of organisms of the sporogenes type in the treatment of wounds in man.

Let us for practical purposes group the vibrion septique of Pasteur, the bacillus of Ghon and Sachs, and whatever organisms resemble them, under the name of "the vibrion septique group." A definition of this group is to be found on page 389, b. May I be forgiven if, solely in reviewing the literature, I sometimes use, in conformity with an author, the term "bacillus of malignant oedema" as applying to a proteolytic strain?

Although the traditional idea has been that B. chauvoei is nonproteolytic and may by this sign be distinguished from B. oedematis maligni, some authors have attributed a proteolytic action to it. Sperry and Rettger refer to the well-known putrefactive organisms B. anthracis symptomatici and B. oedematis maligni. McCrudden states that B. chauvoei putrefies fibrin. Many older authors do not seem to be very clear on this point, or they make no mention of it. It is a misconception exactly like that concerning the organisms of the vibrion septique group as dealt with by many workers.

(*b*) *Gas Production in the Animal Body.*—This has sometimes been referred to as a distinguishing character.

Gas production depends on the amount of bacterial proliferation in the tissues, and is a relative matter for each separate wound infection, depending on the site of the wound, its extent and its isolation, and on the toxicity of the specific organism for the specific or individual host and also on the size of the host. The more toxic a strain is for the host, the less bacterial proliferation will there be before the death of the animal, and the less gas will there be formed. Furthermore, a bacterial process may exist longer in a large animal before producing death than it can in a small one. All strains produce gas in meat medium. I find that, given a uniform technic, strains of the vibrion septique group produce distinctly more gas in the muscles and connective tissue of the guinea-pig than is produced by members of the blackleg group. An exception to this statement is the behavior of the vibrion septique group strain which I isolated from whale muscle (p. 443), which is so toxic that it kills before it multiplies to any great extent, and produces little or no gas in the guinea-pig

and rabbit. I have observed also that far more gas is to be found in the intestines of guinea-pigs dying of vibrion septique infection than of those dying of blackleg infection.

(*c*) *Formation of Chains or Filaments on the Liver of Animals.*—This has often been used as a criterion of distinction between the two groups. The malignant edema or vibrion septique strains form filaments and the blackleg strains do not. I find, however, that the mode of filament production and sporulation after filament production varies with the species of vibrion septique organism, with the dose culture, with the time elapsing before necropsy, and with the species of animal on whose liver the filaments are produced. I have never observed the formation of chains on the liver of animals dying of a blackleg infection.

(*d*) *Appearance of Infiltration and Necrosis.*—The appearance of the infiltration and necrosis (gaseous hemorrhage or serohemorrhagic) in the muscles of the host is also used as a differential character. I find the character and degree of the hemorrhagic exudate and the color of the muscle a good lesion to use when standardizing my technic on the guinea-pig. Blackleg almost invariably produces an intense black coloration in spots or in a circumscribed area in the muscles and connective tissue, while vibrion septique strains do not. The latter organisms are, however, distinctly hemolytic in their action, and it would sometimes be very easy for the inexperienced to mistake the coloration produced by them for that called forth by blackleg. It must also be borne in mind that the production of hemorrhagic exudate is a relative matter, dependent on the specific action of the hemotoxin on the corpuscles of the host and on the amount of bacterial proliferation, which in turn is dependent on the location and nature of the wound or focus, on the degree of the toxicity of the specific organism for the specific host, and on the size and age of the host.

(*e*) *Alteration of the Serous Membranes of the Abdominal Cavity.*—This should always be considered in distinguishing between organisms of the vibrion septique group and those of the blackleg group. The tendency to cause injection of the intestines and of the peritoneum is very great in the case of the former and very slight in the case of the latter. This is in the guinea-pig an almost determinative character.

(*f*) *Presence of the Pathogenic Organisms in the Bile.*—This has been used by a few authors as a means of differentiation. Foth (b, 212) found this a criterion of no value. Wulff (706) found the method wholly unreliable.

(g) Depilatory Effect of the Inflammatory Process.—This is a relative character of a little value. It is dependent on the degree of bacterial multiplication in the skin, and is most marked in proteolytic infections, and least marked or quite absent in highly toxic vibrion septique infections. Blackleg infections often show it, and combined with it is frequently a serous exudate. The animals that die most slowly have the greatest bacterial multiplication and their hair is most easily removable.

(h) Delimitation of the Zone of Infection.—This is not an absolute character. In general, the zone macroscopically affected in blackleg infections is more sharply limited in outline and extent than the zone affected in vibrion septique infections.

(i) Speed With Which Death Ensues.—This varies of course with the dose inoculated. Given the same technic, almost all vibrion septique strains kill guinea-pigs before the usual time taken for death to intervene in the case of blackleg infections. Guinea-pigs infected with a dose of 0.5 c c of a 24-hour

culture of a vibrion septique strain die in about 16 hours, those infected with the same amount of blackleg culture die in approximately 20 hours; but there is great variation in these figures. It must also be borne in mind that a blackleg culture does not contain as many organisms as does a vibrion septique culture.

Many authors mention the *characteristic odor* produced in the animal body by blackleg infections. I have not detected any difference in the odor of guinea-pigs dead of the two diseases, but perhaps my perception is at fault.

(j) Minor Characters Common to Both Types.—Both types of organisms tend to cause profound liver disturbances in the guinea-pigs, which are shown by bile staining of the intestines and frequently by an emptying of the gall-bladder. Both types may cause the formation of hemorrhagic erosions on the stomach lining of the guinea-pig, and both may cause enlargement and injection of the suprarenals. Foth (a, 204) and Wulff (45) state that both types produce ochre yellow foci in the liver of cattle. Meyer (c, 684) found infarcts in the liver of cattle dying of atypical B. chauvoei infection, and (b) isolated vibrion-septique-like organisms from liver foci of cattle dying of an obscure disease in the mountain valleys of California. Both types may invade the pleural cavity and even the lungs; both become septicemic in the guinea-pig, with practically no exceptions, and may be isolated from the heart blood. Both types produce a serogelatinous edema of the connective tissue spaces, and a liquid infiltration of the muscles; they soften somewhat the muscles in which the organisms multiply extensively.

(k) Characters Possessed by Neither Type.—Neither type of organism produces, when inoculated in pure culture, a greenish discoloration of the skin, a foul or putrid odor, tissue disintegration, or a brownish, a grayish, or a yellowish discoloration of the muscles. Neither type produces a gelatinous edema of the connective tissue spaces that retains its gelatinous consistency long after section, nor do they produce an edematous infiltration of the muscles. Though the organisms multiply actively on liver, the phenomenon of "foamy liver" is probably usually a postmortem one.

(l) Pathogenicity for Certain Species.—Pathogenicity for certain laboratory animals, notably for the rabbit, is frequently cited in the older textbooks as a final criterion when one is in doubt as to the nature of an organism under consideration. Malignant edema was thought to be pathogenic for the guinea-pig only in spite of the fact that the bacillus of Ghon and Sachs, generally known to be of the same group as vibrion septique, is not pathogenic for rabbits. Meyer's hog bacillus is relatively nonpathogenic for rabbits, and Meyer (a) emphasizes the fact that "any conclusions drawn from such (pathogenicity) tests would be entirely erroneous without the necessary microscopic examination and anatomic studies." When one considers how greatly the technic of various workers differs in making pathogenicity tests, and when one bears in mind the fact that different individuals of a species vary in their susceptibility to infection, one is ready to agree heartily with Meyer's statement as to the secondary importance of such tests.

(m) Epidemiologic Considerations.—Most deceptive, and, may I venture to say, crudest, of all differentiations between the two groups of organisms are those based on epidemiologic grounds. Diagnosis of the nature of a disease according to whether it is endemic in a locality or whether it is rare in that locality is an unscientific proceeding. Specific diagnosis of an infection, which is based on the presence of a wound as a portal of entry, on the history of a recent parturition, or on the age of the animal attacked, is likewise unjustifiable.

3. CULTURAL AND MORPHOLOGIC DIFFERENCES BETWEEN THE ORGANISMS OF THE TWO PRINCIPAL GROUPS

(a) Motility.—The motility of blackleg and vibrion-septique-like organisms is given differently in different works. Both types (Arloing, Cornevin, Thomas; Pasteur) were originally described as motile. I find that in an open drop on an unwarmed slide the vegetative rods of the blackleg organisms in a 24-hour meat culture may be sluggishly or actively motile, moving in a dancing head-over-heels fashion, while under the same conditions, organisms of the vibrion septique group are noticeably but sluggishly motile, moving occasionally in the manner of a boat or even sidewise, but not tumbling or dancing.

(b) The Gram Stain.—The Gram stain is used to differentiate the two groups. Lehmann and Neumann (500) give malignant edema as gram-negative and blackleg as gram-positive. Bongert says that malignant edema is gram-negative. Friedberger and Fröhner give both as gram-positive. Von Hibler (a, 13) states that all anaerobes studied by him are gram-positive but that the Ghon-Sachs bacillus and the malignant edema bacillus are the most easily discolored by the alcohol. Foth (a and b) declares both types to be gram-positive. My experience runs counter to most of these statements. I prefer a carbol-methyl-violet (6BN) Gram stain[8] with slight decolorization or a thin carbol-gentian-violet Gram stain, which requires longer decolorization. With either of these stains vibrion-septique strains are usually gram positive and blackleg-group strains are gram-negative.

(c) The Resistance of Spores to Heat.—This has been used by von Hibler and by some of his predecessors (a, 217) as an anaerobic character. I doubt if spore-resistance could be used to distinguish blackleg strains from vibrion septique strains—and we have many characters to use for differentiation that are more definite.

(d) Morphology.—Opinions vary greatly as to the value of morphologic characters for the differentiation of blackleg from the vibrion septique group. This confusion I attribute solely to the reigning confusion as to the identity of the strains studied. One reason for this state of affairs is that there still are diagnosticians who call an organism blackleg because it comes from a cow. But another important reason is the existence of a large number of contaminated strains in circulation among laboratories. The usual contaminators of sporogenes and bifermentans type, or of tertius or putrificus morphology, naturally upset all morphologic conceptions. Moreover the blackleg organisms in their growth in muscle resemble the vibrion-septique organisms growing in muscle more closely than either type resembles most other anaerobes. This does not mean that they are alike or indistinguishable.

I may sum up the value of morphologic determination according to my own experience as follows: If the worker is unfamiliar with anaerobic technic and with the anaerobic group as a whole, his opinion (beyond his observation of filaments on the liver of an animal) is worthless. And if the worker has not an eye capable of distinguishing with some certainty a contamination in a smear of an anaerobic culture in vitro or in an animal, his opinion is worthless. But for the trained anaerobist, employing a technic with which he is familiar, the organisms of these two groups are, in morphology and in staining reaction, highly characteristic, and they are not to be confused. I could under-

[8] Two and five tenths c c saturated alcoholic solution of stain in 100 c c of 5% pure phenol; dilute 1: 10, filter, apply for 2 minutes, wash, apply Lugol's iodin 1 minute, wash with 96% alcohol, once across the surface of the slide for methyl violet (8 drops), twice across the surface (12 drops) for gentian violet. Counterstain with dilute 1: 10 carbol fuchsin.

take to teach any observing bacteriologist the difference between the two types in a few hours. I would suggest that those interested in this subject study carefully pure cultures of organisms of the two groups in the guinea-pig and in meat medium with a weak or carefully decolorized Gram stain (see p. 401, b) Forms are occasionally seen which may be referred to either group; morphologic criteria could not free a suspected mixed blackleg-vibrion-septique culture of suspicion, but the general picture of the cultures and of the smears of animal exudates containing these two types is entirely different, and any one can recognize this difference on his own mediums and with his own stain as soon as he is acquainted with both types.

(e) General Cultural Characteristics are not of great value in distinguishing the two groups. In general the organisms of the blackleg group are not so active in their growth on artificial mediums as are those of the vibrion-septique group; they occasionally fail to grow on a medium on which vibrion-septique organisms will readily grow. For example, I have repeatedly failed with massive inoculation to induce them to grow on brom-cresol milk. But in gross biologic determinations the two groups are so closely related that they are thrown together rather than separated. However, gross determinations separate these groups from other groups, especially from the Welch group and from the oedematiens group. Finer biologic determinations may be of great value, not only in differentiating the blackleg organisms from the vibrion-septique organisms, but also in differentiating distinct species within these groups. So far as my experience in the matter goes, the more detailed and more or less quantitative cultural tests, combined with morphologic characters, are of more value in differentiating these organisms than are immunologic tests.

(f) Colony Formation.—Colony formation has not been used by many recent authors as a means of differentiation between the two groups. I have, however, found colony formation in agar under carefully controlled conditions to be of immense value in distinguishing, not only between the blackleg and vibrion-septique groups, but also between the individual species in these groups.

(g) Carbohydrate Fermentations.—Carbohydrate fermentations have occasionally been resorted to as a means of differentiating anaerobes.

Achalme analyzed the behavior of vibrion septique and the bacillus of symptomatic anthrax on sugars. As his cultures were proteolytic I shall not quote his results.

Smith used fermentation tests on three sugars to distinguish his anaerobic strains. The nonproteolytic strains called "Rauschbrand" behaved consistently; the proteolytic strains called "edema bacilli" behaved differently from the nonproteolytic ones and consistently with each other.

Bahr made a more extensive study. He divided his strains into 5 groups, the first group containing 9 strains of quite different origin: sheep, hogs, cattle and a horse; they were from different countries. The other groups contained one strain each. Bahr isolated his strains by animal passage and a colony method.

Grosso studied carbohydrate fermentations. His isolations were carefully made.

Meyer (a) found his hog strain, a vibrion-septique strain, and a blackleg strain to behave alike.

Todd worked out an elaborate scheme for sugar determinations on this group, but some of his cultures were proteolytic and the work cannot be quoted.

TABLE 1

TABLE OF CARBOHYDRATE FERMENTATIONS AS QUOTED FROM VARIOUS WORKERS

	Glucose	Levulose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Mannose	Xylose	Rhamnose	Arabinose	Amylum sol.	Inulin	Cellubiose	Glycogen	Salicin	Amygdalin	Trehalose	Mannite	Dulcite	Quersite	Sorbite	Glycerin	Adonite
Smith's nonproteolytic	+	..	..	—	..	+	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.	..	..	..
Smith's proteolytic	+	..	..	+	..	+	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+	..
Bahr's group I	+	..	..	—	+	+	..	..	—	—	—	..	..	..	..	..	..	..	—	—	..	—	+	—
3 Bradsot																								
2 Oedematis maligni																								
1 Pseudo-Rauschbrand horse																								
3 Rauschbrand cattle																								
Bahr's group II	—	..	..	—	+	+	..	..	—	—	—	..	..	..	..	..	..	..	—	—	..	—	+	—
Rauschbrand, Kitt																								
Bahr's group III	+	..	..	+	+	+	..	..	—	—	—	..	..	..	..	.	..	..	—	—	..	—	—	—
Bradsot, Scotland																								
Bahr's group IV	+	..	..	+	+	+	..	..	+	—	+	..	..	..	..	..	..	..	+	—	..	+	+	—
Rauschbrand, Ostertag																								
Bahr's group V	+	.	..	+	+	+	..	..	+	+	+	..	..	..	..	..	..	..	+	+	..	+	+	—
Malignant edema, Halle																								
Grosso's group I	+	..	..	—	..	+	..	..	—	—	—	..	..	..	..	..	..	..	—	—	—	..	—	—
2 Malignant edema																								
1 Bradsot																								
Grosso's group II	+	..	..	+	..	+	..	..	—	—	-	..	..	.	.	..	..	.	—	—	—	..	—	—
2 Pseudo-Rauschbrand																								
Grosso's group III	+	..	..	+	..	+	..	..	—	—	—	.	..	..	..	..	..	..	—	—	..	..	+	—
Rauschbrand																								
Meyer's hog bacillus	+	..	..	—	+	+	..	..	—	—	—	..	..	.	..	.	..	..	..	—	..	—	—	..
Vibrion septique																								
Rauschbrand 2, Munich																								
Robertson, several Vibrion septiques	+	+	+	—	+	+	..	..	..	..	..	..	—	..	..	+	..	..	—	—	..	..	—*	..
Robertson's B. chauvoei	+	+	+	+	+	+	..	..	..	..	..	..	..	..	..	..	..	..	—	..	..	..	—*	..
Christiansen's whale septicemia bacillus	+	+	+	—	+	+	—	+	—	—	—	—	—	+	—	+	—	+	—	—	—	—	—	—

* Med. Res. Committee, No. 39.

Robertson found vibrion-septique strains behaved alike, and a B. chauvoei strain behaved differently from them.

Christiansen made extensive determinations on his whale septicemia bacillus.

One observes a general tendency on the part of anaerobes of these groups to split the hexoses and the disaccharids, and to leave the higher carbohydrates untouched. Smith's nonproteolytic group, Bahr's group 1 (9 strains), Grosso's group 1, Meyer's 3 organisms, Robertson's vibrion septiques, and the whale septicemia bacillus of Christiansen are wholly consistent, and may be taken as characteristic of the vibrion-septique group. They may be signalized as not splitting saccharose. The remaining strains referred to B. chauvoei are not so consistent in their behavior; Grosso's and Robertson's strains split saccharose. There are not enough data from which to draw further conclusions. I hope to perform extensive fermentation tests with my strains in the near future. It would appear that a satisfactory basic medium for carbohydrate determinations for use with anaerobes is yet to be found.

4. DIFFERENTIATION BETWEEN THE TWO PRINCIPAL GROUPS BY MEANS OF IMMUNE REACTIONS

(*a*) *Immunization in General.*—The anaerobes with which we are dealing are antigenic organisms par excellence. Immunization experiments have been carried out by many workers, in almost as many ways. Results are apparently contradictory, but on careful scrutiny they are found to be consistent. Immunization of guinea-pigs has been accomplished with cultures, washed or heated organisms, and organisms chemically attenuated; with toxic and even nontoxic filtrates of cultures and of infected tissue; also with serum from immunized animals.

(*b*) *Active Immunization with Cultures.*—We shall deal here chiefly with immunization work which was performed with the purpose of distinguishing our two important types. I find the following notes as to the active immunization of laboratory animals by means of cultures:

Roux in 1888 used culture which had been heated at 115 degrees for immunization purposes.

Kitasato in 1889 found that a two-weeks-old culture of B. chauvoei in guinea-pig broth could be used for the immunization of guinea-pigs, as could a heated culture.

Le Clainche and Vallée found that guinea-pigs immunized by attenuated blackleg virus were not immune to vibrion-septique infection.

Markoff used cultures in 0.5% sodium formate broth with 2-5% glucose and found immunization of guinea-pigs with blackleg and chain-forming organisms possible with such cultures.

Wulff (612) used dry powdered infected muscle for immunization.

The authors referred to above all agree that blackleg and the chain-forming organisms may be distinguished by this means.

(*c*) *Types of Toxin.*—The best known anaerobic toxins are those of the proteolytic organisms B. tetani and B. botulinus. These toxins are apparently produced slowly in a medium, and increase in power as incubation continues. They are pathogenic in extremely minute doses. It is of interest that the more proteolytic a pathogen is, the more powerful is its toxin, the less

energetic its invasive properties. The toxin of B. oedematiens, which has mild proteolytic properties (Med. Res. Comm. No. 39, 63), is less powerful than that of B. tetani and that of B. botulinus which is the most highly proteolytic of the three, but it is far more powerful than that of the vibrion-septique and Welch-group types, which are nonproteolytic organisms (Med. Res. Comm. 39, 116).

(*d*) *The Aggressin Test.*—The determination of toxin and antitoxin relationships is of immense practical value. Tulloch has shown in his tetanus work that aggressins are antigens which must also be considered in anaerobic infection. Probably cultural methods can be made to supersede the agglutination method entirely, because immunologically it has no practical significance. But the toxins and the aggressins have great practical significance: What does it matter to the therapeutist if two strains can be shown to belong to two different species by colony formation or other delicate methods, so long as they produce the same toxin and aggressin? A practical method of arriving at the combined toxin-aggressin factors would be by means of the immunization of guinea-pigs with young whole culture, after a preliminary inoculation of killed organisms. More delicate methods of demonstrating aggressins are two: (1) The delicate phagocytic methods in use in Sir Almroth Wright's laboratory may be used to show the action of bacterial products and their antibodies on leukocytes; or (2) accurate animal experiments may be turned to, filtrates of definite age from mediums of standard composition being used.

Immunization by means of filtrates was undertaken in 1888 by Roux, who found that filtrates of cultures of vibrion septique and B. chauvoei were excellent for immunizing guinea-pigs. He found filtered serous exudate of guinea-pigs dead of vibrion-septique infection to be more highly toxic for guinea-pigs than that of guinea-pigs dead of blackleg infection, and he found such serosity to be a good immunizing agent. By such a means he immunized guinea-pigs against both diseases and found that those immunized against vibrion septique were not immune to symptomatic anthrax, but that those immune to symptomatic anthrax were immune to vibrion septique. Kitasato repeated Roux's experiment and found that Rauschbrand did not protect against malignant edema.

Schöbl (1910) found that centrifuged edema fluid of an animal dead of blackleg infection immunizes guinea-pigs against blackleg inoculation. This method has received wide practical application in the immunization of cattle.

Nitta (1918) found that blackleg culture filtrate furnished adequate protection to cattle.

(*e*) *Passive Immunization.*—More extensive work has been done in the way of differentiation by means of passive immunization.

Duenschmann, in 1894, attempted to demonstrate immunologically the relationship between vibrion septique and B. chauvoei. His immunization strain, called B. chauvoei, which he received from Arloing, who used it for vaccine, was, however, a member of the vibrion-septique group. This was pointed out by Le Clainche and Vallée (595). Duenschmann considered it to be a true B. chauvoei because it did not kill rabbits. It killed guinea-pigs rapidly "with all the well-known signs of symptomatic anthrax" (details not given); it sporulated heavily in the animal body, many spores being median; the bacilli were short, but formed long motile filaments on the peritoneal wall. Duenschmann found that the serum of rabbits immunized with blood cultures of this strain immunized guinea-pigs against a stock vibrion-septique strain of the Pasteur Institute.

In 1900 Le Clainche and Vallée studied immunologically the relationships between B. chauvoei and vibrion septique. They found that a B. chauvoei serum which protected guinea-pigs against B. chauvoei inoculation, would not protect against several strains of vibrion septique, all of which were protected against by one vibrion-septique serum.

Foth (a, 254) used serum of rabbits immunized by injection of cultures of Rauschbrand bacilli. This was given subcutaneously to guinea-pigs in one thigh, culture was given on the other; and animals that were protected, while controls died with lesions of Rauschbrand, were considered to prove the existence of Rauschbrand.

Kitt (a) says that serum immunization may be used to differentiate Rauschbrand from the infections that simulate it.

Markoff preferred serum immunization of guinea-pigs to culture immunization, and found that it divided his organisms into fundamental groups.

Wulff (612) found serum immunization a diagnostic aid of great value.

Differentiation of blackleg from malignant edema by means of toxin-antitoxin determination has been undertaken by Grassberger and Schattenfroh (b and c). They hold this procedure to be the only fundamental means by which to distinguish the two types from one another, which is entirely comprehensible when one considers their technic. They use an inoculum of dried juice from an animal dead of blackleg, which is confessedly impure, which Grassberger and Schattenfroh hold to be unimportant. They then place a piece of this material in a tube containing a chunk of "sterile" beef muscle, taken aseptically from a larger piece (Foth [b, 241] and Conradi claim that such muscle is seldom sterile), pour over it 2-3% glucose broth, incubate in a Buchner tube and in 24 hours find that microscopic examination shows sporulating and nonsporulating forms of "half denatured" Rauschbrand bacilli! They find that this broth is highly pathogenic (unfiltered?) for rabbits, and that a highly potent antitoxin will protect the rabbits. Likewise, malignant edema forms a toxin which is specific.

I have every confidence that the toxins of blackleg and of vibrion-septique strains will be found to be specific—what we need to know is whether all toxins produced by the members of one group are alike (see p. 427). I wish, however, most emphatically to protest against such gross methods as those of Grassberger and Schattenfroh. Immunologic work cannot be regarded as serious when it is performed with impure cultures. It is especially necessary to have pure cultures with which to produce immune serums, but Grassberger and Schattenfroh do not mention any precautions taken in this matter. Especially is this fact to be considered as entirely disqualifying the work of Grassberger and Schattenfroh when one considers the fact that the usual "denatured form" of Rauschbrand described by Grassberger and Schattenfroh is the ubiquitous B. Welchii (B. phlegmones emphysematoseae [von Hibler a, 5]), and that that organism produces a highly potent toxin in the type of medium described by Grassberger and Schattenfroh.

Meyer (a) found that serum immunization sharply distinguishes the groups.

Nicolle, Cesari, and Raphael demonstrated by means of antiserum the identity of the toxins of various tissue-invading strains of animal origin. They found also that their antiserums protected against cultures of the various strains. These strains comprised one strain from the blood of a cow, which was putrified, one from the cadaver of a rabbit "altéré," and two from the cadavers of guinea-pigs "altérés"—these strains Nicolle, Cesari, and Raphael called vibrions septiques. The authors studied also several strains that they call B. chauvoei, one of which came from a hog and the others from cattle

I can find, however, no evidence in their protocols that they worked with any typical strains of B. chauvoei. They repeatedly insist that their strains all behave alike except for some differences in pathogenicity, the organisms all kill rabbits if the dose given is as much as 1 c c, and all apparently produce "bulles gazeuse" in the animal body, and congestion of the intestines; the black hemorrhagic exudate so typical of blackleg is not mentioned. It is impossible to believe that differences so striking as those noticed in the pathology of the two diseases in the guinea-pig body would have been passed over by these workers had they been seen. The identification of the strains of hog and cattle origin was probably referred to the donors of the cultures. Three of these strains were from Dr. Vallée. I find that one of Vallee's classic B. chauvoei strains belongs to the vibrion-septique group. I am at a loss to account for this as it is apparent that Le Clainche and Valee were entirely aware of some of the distinctions between the two types. Le Clainche and Vallée state, however, that it is difficult to keep a strain of B. chauvoei from becoming displaced by vibrion septique through animal passage. I have never known this to happen with my technic, though it is quite conceivable that it might happen if peritoneal or subcutaneous serous exúdate were used for purposes of inoculation.

A recent piece of work is that of Klose (c). Klose studied the protection afforded to mice and guinea-pigs against the toxins of anaerobes by antitoxic serums. The purity of Klose's strains would bear investigation. He divided them into two groups, the putrificus (proteolytic) group, and the Rauschbrand group; in the latter are included all strains which formed a toxin that was neutralized by a "Rauschbrand" serum of the "Höchster Farbwerke," and also the organisms which culturally resemble the Ghon-Sachs bacillus. His results show that the Höchst "Rauschbrand" serum must have been made from a vibrion-septique strain. Klose investigated 4 strains and 5 serums. Two strains were distinctly proteolytic (K16b and KI); one (Ficker I) partially liquefied serum but was relegated to the nonproteolytic group, and one was nonproteolytic (Berlin). The serums "Gas-Oedem Höchst Op. 3," "Rauschbrand Höchst," and "Goldfuchs" from v. Wassermann, all protected against the nonproteolytic strain, the slightly proteolytic strain, and one of the highly proteolytic strains. They did not protect against the highly proteolytic strain KI, and the antitoxin of strain KI did not protect against the other strains. The pathogenesis strain KI may be assigned to the oedematiens group, as it resembles B. novyi (a). Landau showed that the Höchst serum did not agglutinate two of Kitt's Rauschbrand strains. I doubt if any true Rauschbrand entered this complex. The matter of proteolysis I lay to contaminations. Toxin determinations will probably prove to be excellent for the practical differentiation of these organisms.

Klose (a) studied elaborately the toxin of a highly pathogenic anaerobe that resembles the B. oedematis maligni II of Novy. He insists (b, 293) that immunologic methods, especially toxin-antitoxin determinations, are fundamental criteria for specific determination of anaerobes. Zeissler (a, 489) finds Klose's strains of pathogenic anaerobes contaminated with proteolytic organisms and attacks Klose for placing "putrificus" strains in the Rauschbrand group on the basis of toxin-antitoxin determinations. Zeissler calls the value of toxin-antitoxin determination seriously into question. Of course, if one is to use impure cultures in such determinations one should not make the mistake made by Klose of confounding the cultural characters of the contaminators with those of the toxin-forming organisms.

Various workers have produced toxins from the organisms of the groups under discussion, though Haslam and Lumb showed that blackleg culture filtrates made by four different laboratories possessed immunizing properties but no toxic power for the guinea-pig. These authors were careful as to the identity and purity of their cultures. I should not consider that their work shows that blackleg organisms do not produce toxin, since the cultures used were not young ones.

(*f*) *The Agglutination Test.*—The agglutination reaction has naturally frequently been utilized as a means of differentiating anaerobes.

Le Clainche and Vallée sharply differentiated B. chauvoei from vibrion septique by means of the agglutination reaction.

Hillbrand differentiated bradsot from malignant edema by agglutination.

Grosso discusses the question. He found that all bradsot strains were agglutinated by the same monovalent serum, which also agglutinated all his malignant-edema strains. He therefore pronounced the malignant-edema organisms to be the cause of bradsot. His agglutinations were consistent, as follows:

Bradsot serum agglutinated malignant-edema cultures.
Malignant-edema serum agglutinated bradsot cultures.
Rauschbrand serum did not agglutinate bradsot cultures.
Rauschbrand serum did not agglutinate malignant-edema cultures.
Malignant-edema serum did not agglutinate Rauschbrand cultures.
Malignant-edema serum did not agglutinate pseudo-Rauschbrand cultures.
Rauschbrand serum agglutinated Rauschbrand cultures.

Grosso defines as pseudo-Rauschbrand a type of organism that resembles Rauschbrand closely and is pathogenic for mice; it does not form chains. Grosso isolated his organisms from the heart blood of guinea-pigs.

Markoff used the agglutination reaction to distinguish his strains which were of cattle origin. The filament-forming strains fell into one group and the nonfilament-forming ones into another.

Wulff (612) found the agglutination of cultures to be of value in distinguishing Rauschbrand from diseases that resembled it. The serum of cattle dead of Rauschbrand contains, however, no agglutinin.

Meyer (a, 458), made use of the agglutination reaction for the identification of his hog bacillus. The serum made with the hog bacillus agglutinated to a high titer a vibrion-septique strain of the Pasteur Institute and to only a low titer the blackleg and malignant-edema strains which were available.

McIntosh found that the agglutination reaction distinguished sharply between the groups of anaerobes. A B. chauvoei serum agglutinated its own strain to a high titer and a vibrion septique strain to a low titer, and the serum of the vibrion-septique strain agglutinated the homologous strain to a high titer and the B. chauvoei strain to a low titer.

The Anaerobe Committee recommend agglutination as a means of distinguishing the groups of organisms.

Robertson (b) found that the vibrion septique strains studied by her formed four groups on the basis of the agglutination reaction and she found that impure or recently isolated strains frequently failed to agglutinate. She considers a positive agglutination with a vibrion septique serum to be specific.

Zeissler (a, 110) asserts that he has found Rauschbrand 10 times as a human wound infection. Agglutination with a serum derived from a "Rauschbrand" (?) strain from a cow was the determinative measure used. (In 6 of these cases Fraenkel's bacillus was also present.) Zeissler does not say

whether the wound infections were gangrenous processes, or whether he isolated the organism merely from septic wounds. He says (b, 40) that the Ghon-Sachs bacillus so closely resembles the Rauschbrand bacillus that there is no fundamental or morphologic difference between the two, except that the former makes filaments in the animal body. As Zeissler worked with a colony method that should have distinguished true Rauschbrand from Ghon-Sachs-like organisms, I am forced to suspect that his "Rauschbrand" used for the manufacture of the agglutinating serum was an organism of the vibrion-septique group that showed little tendency to form filaments in the animal body, or perhaps that Zeissler did not look at smears from the peritoneal cavity to find filaments. This suspicion mounts almost to certainty because Zeissler found the bacillus of Ghon and Sachs only once in man and "Rauschbrand" 10 times.

Zeissler (a, 110) states that two anaerobe strains, which were manifestly of a different species from that with which a serum was made, were agglutinated by that serum. He guarded against auto-agglutination. He heartily condemns the agglutination reaction as a means of differentiation of anaerobes and calls into question all decisions that rest on it.

Passini finds that putrificus-immune serums may agglutinate organisms of another species to a higher titer than that to which they agglutinate other strains of putrificus. He intimates that this finding strengthens the thesis of Grassberger and Schattenfroh, namely, that the anaerobes are all more or less the same. I should suggest, instead, that the data of Passini show the worthlessness of the agglutination reaction, at least as practiced by him.

Robertson (private communication) immunized a rabbit with a strain of my own (B. II, Hempl) that is to be assigned to the bifermentans group. The serum of this rabbit agglutinated B. sporogenes. I do not think there is any reason to doubt the purity of the strain used by Miss Robertson.

My experience with agglutination in the differentiation of anaerobes has been distinctly unsatisfactory. I heartily agree with Zeissler in his condemnation of it. The phenomenon of auto-agglutination interferes frequently: in the oedematiens group it makes all agglutination work impossible; in the blackleg group it often gives trouble but may be eliminated by adjusting the electrolyte content of suspensions and by adjusting the P_H reaction; in the vibrion-septique group it causes little inconvenience. But there is a variable element in the reaction, existent in the cultures of the organisms to be agglutinated, which lies always ready to trap the unwary. I find that vibrion-septique strains fall into several groups on agglutination, as stated by Robertson (b); but when the experiment is repeated most of the strains will behave as they did before; one or two of them may behave, however, in so different a fashion that the worker is at first led to suspect that he has made mistakes in his technic, and is later forced to conclude that the medium and technic used are unsatisfactory for the problem in hand. The medium used by myself was a casein-digest broth containing a chunk of liver. All digest mediums have now been discarded and others are being tried. It is to be hoped that a technic and medium may be developed that will furnish regularly reproducible results. With a given serum some strains agglutinate to a high titer and some to a low titer. The phenomenon of an inhibition zone was also observed. Several strains are agglutinated by one serum while another serum agglutinates only one of them. One strain was agglutinated by serums of the three principal types. The agglutination reaction may in general be used to separate one group from another, but we have plenty of other criteria for such separation that are easier to use and more dependable.

The Medical Research Committee (39, 55) term the agglutination reaction "ultra specific." For the therapeutist this statement is probably correct, but for the systematist and for the epidemiologist the finer differentiations are of great value. It is to be hoped, therefore, that absorption-of-agglutinin tests may lead to some advance in breaking up the groups of anaerobes. Agglutination tests in the anaerobic group are not to be undertaken lightly with a few strains, and I do not believe that agglutination tests performed with impure cultures can be of value, at least not in the vibrion-septique and blackleg groups. It should be remembered that the use of another much-heralded immune reaction, namely phagocytosis, has been largely abandoned because of occasional inexplicable inconsistencies in its behavior.

(*g*) *The Precipitin Test.*—In large veterinary institutes in countries like Germany, where losses by Rauschbrand are recompensed by the government, the problem of determining the nature of such a disease cheaply and expeditiously from pieces of flesh sent to the laboratory is a serious one. Miessner and Lange propose the precipitin reaction as a solution for such a problem. They successfully immunized horses to Rauschbrand and to malignant edema. The precipitin reaction was carried out on the juices of the muscle of the animal in question. It failed or gave an equivocal result in 14 out of 65 cases that were anatomically and microscopically referable to Rauschbrand. But Miessner and Lange do not tell us what results these cases gave with a malignant-edema precipitin, nor do they give us other details as to the strains of organisms found in these cases. I think it very likely that the material that showed no precipitation with a Rauschbrand serum may have been from animals with an infection by an organism of another group, and thus worth investigating. One point must be suggested: Perhaps precipitin reactions on extracts from pieces of Rauschbrand muscle will work best for low virulent infections and less markedly for highly toxic infections because such reactions are probably dependent on the number of bacilli in the muscle. Miessner and Lange note great variation in the intensity of the reaction.

Ascoli and Valenti found the precipitin reaction of great use in the diagnosis of anthrax. Pfeiler says that anthrax is the disease par excellence whose diagnosis may be established by this reaction. He quotes several authorities who used it for medicolegal determinations on badly decayed cadavers that were entirely unsuited to microscopic or bacteriologic examination. He and Rehse obtained positive results with a sample of mud from a spot where a cow, sick of anthrax, had been slaughtered weeks before.

(*h*) *Hemolysins.*—Nicolle, Cesari, and Raphael showed that the hemolysins in 5-day Martin's broth cultures of their strains showed variations which were of no differential value. I find all organisms of the tissue-invading groups highly hemolytic in their action in blood broth.

(*i*) *Hemagglutinins.*—The same authors found that all these strains gave hemagglutinins for guinea-pig red blood cells, and that four of their B. chauvoei strains and one vibrion-septique strain gave hemagglutinins for rabbit red blood cells.

(*j*) *Leukocidins.*—Eisenberg found that a Rauschbrand antileukocidic serum was as effective against the leukocidin of B. oedematis maligni as against that of B. anthracis symptomatici. Eisenberg does not present details as to the identity of his strains.

(*k*) *The Complement-Fixation Test.*—Rocchi found complement fixation to distinguish anaerobes of various strains. Even different strains of the same name were not identified by this reaction. The collection was one of strains from various laboratories, and details as to their behavior are not given.

Ascoli and Valenti could find no application for the complement-fixation reaction in anthrax material.

(1) *Summary of Uses of Immunologic Tests.*—It may be concluded that for separation of the organisms of the blackleg group from those of the vibrion-septique group various immunologic methods may be employed. I should place these in order of preference with regard to convenience and value as follows: Immunization of guinea-pigs by serum, by toxin, by cultures; precipitation; agglutination. I should hesitate to classify the other methods.

I venture to say that immune reactions will in time automatically be relegated to the province of the serum laboratory where the antigenic status of the organism is its only interesting feature. We have or can develop abundant other methods for classifying the anaerobes according to their group affinities, and we have cultural methods that divide up the groups into smaller and more readily recognized subdivisions than do immune reactions.

II. AN INVESTIGATION OF MATERIAL DERIVED FROM ANIMALS

By isolating organisms from animal material and from cultures derived from animals and by securing strains already isolated by others I have made a collection of 54 strains of pathogenic tissue-invading anaerobes. A classification of these strains according to their group affinities and according to their source is excerpted from the appendix.

1. PATHOGENIC STRAINS DERIVED FROM ANIMALS

Strains of the Vibrion-Septique Group 30: from cattle 13, from sheep 8, from horses 2 (one spontaneous infection, one accidental laboratory infection with a human strain), from hogs 2, from guinea-pigs 2, probably from guinea-pigs or rabbits 2, from whale 1.

Strains of the Blackleg Group 21: from cattle 19, typical, 15, atypical (probably none identical) 4, from sheep 2, one apparently typical and one atypical and unique.

Organisms of the Oedematiens Group, 3: from horses. Three edema producing organisms of the same species referable to the oedematiens group were specifically distinct from all other strains in my collection.

From a few specimens of original material from cattle and sheep I was unable to isolate pathogenic organisms—the material was probably too old. I much regretted this in the case of the sheep material from Professor Hamilton which was given me by Dr. Jensen, because Hamilton's material is in a way classic and has been studied by several workers. From a sadly large proportion of cultures it was found to be quite impossible to isolate a pathogenic organism. Proteolytic contaminations had completely outgrown the original pathogen. Still, a fairly large number of pathogenic organisms was recovered.

2. TECHNIC OF ISOLATION

The technic employed in isolation was, briefly, as follows:

Muscle material or culture received was inoculated into tubes of meat medium and was incubated anaerobically for about 20 hours. It was then examined. If the flora comprised aerobes, the culture was heated in a pipet and inoculated into fresh medium. If, on examination, the flora was apparently highly proteolytic, a large dose of 1.5-2 c c was injected into the thigh muscle of a guinea-pig; if the flora was nonproteolytic and suggested the presence of a known pathogen, a small dose of about 0.5 c c was injected into the thigh muscles of a guinea-pig. To avoid contamination of syringes by sporulating organisms, Pasteur pipets have been used for inoculations. This is the method used by von Hibler. Cultures were recovered from the heart blood after the death of the animal and were studied critically to detect contaminations. Further isolation was accomplished early in the period of the investigation by Barber's single bacillus technic (usually more than one bacillus from an apparently pure culture being used), and later by my usual deep colony method. All cultures were checked several times for purity by a deep colony method. When a culture with a history of pathogenicity was found to be nonpathogenic for a guinea-pig, it was inoculated (20-hour culture) into another guinea-pig with a small drop of lactic acid. If it then failed to infect, it was inoculated onto liver broth and the same technic was employed as for meat medium. To this type of perseverance I owe the unique and highly pathogenic strain AS. If no pathogenicity was shown after these efforts, the material was abandoned.

An interesting feature was that in three cases in which a mixed infection of a vibrion-septique type of organism and of a blackleg type of organism existed in the material from the original cow, as could be easily detected by the morphology of the organisms in the meat medium, a culture of the mixture on meat medium gave a vibrion-septique infection in the guinea-pig, and a culture on liver broth gave a blackleg infection in the guinea-pig. This could probably not be accomplished with all such mixtures, as vibrion-septique strains are known to differ considerably in pathogenicity. I know, however, of no other way, short of immunization of guinea-pigs, whereby blackleg may be isolated when a vibrion-septique strain is present.

This is an expeditious method for isolating pathogens. I do not pretend that I isolated all the pathogenic organisms in the material at hand. It is, however, probable that all the vibrion-septique strains present were isolated. I know of no cultural method of isolating blackleg from a gross mixture. Its colonies are so slow to develop that it would seem almost a hopeless task to try a colony method to isolate it unless it were in predominance. Vibrion-septique organisms may, however, easily be isolated from mixtures by a colony method.

3. DISCUSSION OF TECHNIC OF ISOLATION AND OF SOURCE OF MATERIAL

I am well aware of what some of my critics will say concerning this method of isolation. Foth emphasizes repeatedly that carefully dried muscle of animals dying from blackleg should be the material used in inoculating the guinea-pigs by whose aid the primary isolation is made. Otherwise, says he, contaminating organisms may also infect the guinea-pig. Contaminations are of three kinds: nonsporulating organisms, which I always kill by heat before the culture used for inoculation is grown; sporulating organisms capable of penetrating tissue in the company of another organism only; and sporulating organisms of high

pathogenicity. The two latter groups Foth lumps in general together as "verbandbildende Bakterien." If one has only one guinea-pig to use per strain it would be unwise to give it a heavy dose of mixed anaerobic culture because proteolytic forms might reach the heart blood and the culture therefrom be impure. But may I assure the reader that the element of proteolytic contamination is one with which I am so thoroughly familiar that I should never fail to recognize it and to purify further any culture in case it existed. It would be impossible for me to mistake a mixed blackleg-proteolytic infection or a culture from such an infection for "malignant edema." I think that a great many mistakes may be traced to the fact that the blood of an infected animal usually does not contain enough organisms for the inoculation of animals or even for reliable colony work, and therefore transudates have been used for inoculation purposes. But if one take the heart blood of an animal dead from a blackleg infection or an infection by a member of the vibrion-septique group, inoculate it into a tube of meat medium, and incubate the tube anaerobically over night, one will invariably secure the principal invader, usually, but not always, in pure culture. It never pays to use subcutaneous serosity or peritoneal or pleural transudate for cultural purposes. One should beware of the fact that certain other types of anaerobes, notably B. oedematiens and B. Novyi, frequently kill guinea-pigs without becoming septicemic, and must be obtained from invaded muscle or from edema fluid; from undiagnosed infections, therefore, cultures should be made from various regions.

The critic will say, however, that, given a mixed culture of blackleg, vibrion septique, and a proteolytic organism, I might eliminate quite satisfactorily the proteolytic organism and lose also the blackleg strain because of the presence of the more highly pathogenic strain of the vibrion-septique group. I confess that this might happen and that it may have happened in the series of strains here presented. But if the blackleg strain and the vibrion-septique strain were present in abundance, as they usually are in material from an animal that they have killed, I should emphatically assert that they would both easily be detected in the guinea-pig, pathologically and microscopically. I know my critics will say that this is impossible, but not only am I entirely confident that it is possible, but I have three cases to prove it. I should remark first, that no culture ever passes my hands for inoculation purposes without microscopic examination of a Gram stain made from it; second, that careful necropsy examinations are made of all animals, their lesions noted, cultures taken from the heart blood and from infected muscle far from the site of inoculation, and smears or impressions made from the site of inoculation, inguinal region, axilla, peritoneal wall, liver surface, and from organs with unusual lesions. These smears are stained by Gram's method and carefully studied by myself. I have so studied every animal—over 200 in number—that has died of an anaerobic infection in this laboratory, and I should decline to accept the criticisms of one who was not in the habit of making like careful observations. Four times in the study of my material from cattle I have thought blackleg and an organism of the vibrion-septique group to be present in a guinea-pig infection. Three times I succeeded in isolating two strains—a blackleg strain and a strain of the vibrion-septique type—from the material. Each time I had thought blackleg to be present when examining the original culture which was used for inoculation. The fourth time a vibrion-septique strain alone was present (strain AS from a badly contaminated culture). It is an organism that forms spores lying sidewise in the bacillus and it forms large spoon-shaped orgonts; but otherwise it is to be assigned to the vibrion-septique group. I should hold that, had a cow died of a mixed vibrion-septique-

blackleg infection, and were the two organisms present in anything like equal proportions in the material given me, I should hardly fail to detect both infections.

But my critics will say: "Suppose a cow died of blackleg and an organism of the vibrion-septique group contaminated the material—would you then not be likely to assign your case to mixed infection when the infection was truly monospecific?" The critics would be right. But the interpretation to be made of such a possibility should be on the basis of a true understanding of the chances of such a coincidence. It would probably be somewhat more likely that a vibrion-septique strain might contaminate blackleg material than that blackleg should contaminate vibrion-septique material. But the members of the vibrion-septique group are by no means ubiquitous in their distribution. Carl (c, 869) reviews this subject. Cornevin found the vibrion-septique type of organism rare in forest earth. Jensen found malignant edema rare in earth from various sources in the vicinity of Copenhagen. Feser infected only 7 of 30 rabbits inoculated with earth from various filthy places; among his samples was earth from the vicinity of anthrax cadavers. He does not tell us how many of his invasions were real infections of the vibrion-septique type. Carl concludes that the "malignant edema bacillus," under which name probably are included strains which are not of the vibrion-septique group, is widely distributed, but that it must not be regarded as a ubiquitous organism. We must also remember that we have no proof that the blackleg type of organism is not almost as abundant in distribution as are the organisms of the vibrion-septique group. The blackleg organisms are not pathogenic for many animals, but both types are highly pathogenic for cattle, and cattle are frequently attacked by blackleg. It is not even proved that blackleg organisms are regional in their distribution because blackleg is usually a regional disease. It is more than probable that some factor other than the mere presence of the organism induces infection, and it may be that such factors, rather than the bacillus, are regional in occurrence.

Another point indicates that my strains are not to be taken as grossly misrepresenting the flora of cattle and sheep infections. Guinea-pigs are subject to invasion by various soil organisms; yet I have found no strain from cattle material which was capable of invading guinea-pig tissue on its own initiative which does not fall into one of the two classic groups of cattle invaders. The nonsporulating tissue invaders are, I admit, to some extent eliminated by my technic, but nevertheless, had specifically abundant and gross contaminations of my material taken place before it reached me, other organisms would have been found, as one finds them any day in soil, which would have been pathogenic for guinea-pigs.

As to the material which came into my hands in the form of cultures: For the original mode of isolation of this I can take no responsibility. I place particular importance on that derived from cattle because it was called blackleg by someone else, presumably by the veterinarian who diagnosed the case in the original animal or by the bacteriologist who first studied the organism. It is possible that in one or two of my cases an original blackleg may have been supplanted by a vibrion-septique organism, but to my mind it is improbable that it happened in many cases.

4. STRAINS ISOLATED FROM CATTLE MATERIAL

From cattle material there were isolated 19 strains of anaerobes that are referable to the blackleg group and 13 strains referable to the vibrion-septique group. Three mixed blackleg-vibrion-septique infections were recognized.

Though I regard these findings as of great importance, I do not wish to be understood to present these figures as exactly representative of the relative frequency of these infections in cattle. There are several points that make them ineligible as statistics.

First, they are insufficient in number and they represent scattering cases from the United States and a few from Europe.

Second, their history is varied and inaccessible to me.

Third, some of them have been selectively weeded out by persons cognizant of the difference between blackleg and vibrion-septique infection—Eichorn's strains which he used for vaccine, for example, were all five referable to the blackleg group.

Fourth, a worker would be somewhat more likely to fail to recognize the presence of a blackleg strain than that of a vibrion-septique strain in an old and partially run out culture, because B. chauvoei is a less active grower and less pathogenic for the guinea-pig than the organisms of the vibrion-septique group. Likewise, blackleg would probably more frequently be entirely lost from cultures because of its delicacy.

Fifth, there is probably a greater chance of contamination of cultures and material by vibrion-septique organisms than by organisms of the blackleg group. This element, though it exists, is not, to my mind, of great importance. I hold that such contamination may have taken place in my material, but that it is unlikely that it often did so. A prime point of evidence in support of this opinion is the fact that the four mixed infections (one from sheep), recognized by me, came from original animal material, and the fact that less than a third of the older cultures contained a pathogen. Were contaminations an important source of vibrion-septique strains, the older cultures would contain a large number of such strains.

The following table may be of interest:

TABLE 2

STRAINS ISOLATED FROM THE CATTLE MATERIAL STUDIED

	Animal Material, 14 Samples	Brain Cultures, 7 Samples	Agar or Gelatin Cultures, 23 Samples
2 pathogens isolated	3	0	0
Blackleg group	3		
Vibrion-septique group	3		
1 pathogen isolated	11	7	7
Blackleg group	11	3	2
Vibrion-septique group	0	4	5
No pathogen isolated	0	0	16

The agar and gelatin cultures may in general be taken to have passed through more inoculations and generations than the brain cultures, which were handled by workers more familiar with anaerobes than those who used agar and gelatin cultures. It will be noted that the handling and transplanting of anaerobe cultures, a process which tends always to promote contamination of such cultures, tends also greatly to weed out pathogens and to replace them with nonpathogens. This finding is fully substantiated by a study of human material and cultures from human material. The pathogens may be regarded as rare and as infrequent contaminators of other material. I have only once discovered a vibrion-septique strain as a contaminator of a culture when there

was no record of the presence of an invading pathogen. The culture was a stock tetanus strain of whose previous history I am ignorant. I do not think that loss of virulence is to be considered in connection with these figures. Vibrion-septique strains apparently do not lose virulence; they are among the most highly pathogenic of organisms. It will be noted that strains of the blackleg group preponderate greatly over the vibrion-septique strains in original material from cattle. This might be taken as an indication of contamination of cultures by vibrion-septique strains when they are subject to laboratory processes; but it must be borne in mind that the majority of the muscle samples received were from laboratories that used the strains for the making of vaccines, and they thus were subject to a deliberate weeding-out process. That in old cultures vibrion septique should be the most frequent form may or may not be wholly due to the fact that B. chauvoei is more delicate and more easily lost.

I must, however, say frankly that I should hesitate to conclude from the above figures and to assert emphatically that vibrion-septique infections were common in cattle, were it not for the fact that I feel that the accounts in the literature support me in such a conclusion, and that the close analogy between the infections of cattle and those of horses and sheep and hogs points to this probability. Countless evidences in the literature bear me out in my contention that strains of the vibrion-septique group are frequently isolated and identified as blackleg. Again and again one finds them—many have been quoted in this paper and many more will be observed on more careful search. Examples of these mistakes may be found with like frequency in various countries, and the errors have been made both by pathologists and by trained bacteriologists. They were made many years ago and they are made today. Only a few workers, all of them men who have themselves handled numbers of strains, have seen the light on this subject. Some of them worked when bacteriology was young and some have worked more recently. They express themselves, their work becomes classic, and then it is promptly disregarded in its principal points by the rank and file.

I feel justified in charging the majority of the members of the veterinary and bacteriologic professions who have dealt with the subject, with having misunderstood the etiology of the anaerobic invasions of cattle. They must either give up the habit of assuming that the lesions hitherto diagnosed as "blackleg," or considered as characteristic of blackleg, may be so diagnosed without differential bacteriologic examination; or they must completely relinquish, in connection with the conceptions "Rauschbrand," "symptomatic anthrax," and "blackleg," any idea that these names describe a disease sui generis, and must deliberately include under these names infections by the members of the vibrion-septique group.

5. STRAINS ISOLATED FROM SHEEP MATERIAL

I have isolated from sheep material two strains of organisms that are referable to the blackleg group. Both appear to have been the only pathogen present in their respective specimens. One, a strain apparently resembling in all respects the majority of those from cattle, was isolated from sheep material sent me by Dr. Hadleigh Marsh of Montana. He had diagnosed the case as blackleg. The other, from Halle, Germany, was derived from bradsot material sent me by Dr. Jensen; it is definitely referable to the blackleg group, but in morphology and in colony formation it differs from all the other strains under observation.

How much light my investigation of bradsot material will throw on the subject of sheep infections I do not know. The material studied was all from

cases that had been diagnosed as "bradsot" by different workers in different countries. It is therefore parallel to my cattle material which had all at some time been diagnosed as "blackleg." I have to thank Dr. C. O. Jensen of Copenhagen for all the samples studied. They were, with one exception, pieces of muscle from what I understand to have been the original sheep. There were 11 samples: from 3 I could isolate no pathogen, from 1 the blackleg-group strain RG was isolated, and from 7 samples strains of the vibrion-septique group were isolated. One specimen from Sletten, Norway, furnished two species of vibrion-septique type. They differ markedly in colony formation and in pathogenic action on the guinea-pig, one producing on thigh inoculation the most marked gastritis observed in this laboratory, with a bright red coloration over the whole external stomach surface; the other producing a relatively small amount of injection in the abdominal cavity. These 8 strains are all definitely to be included in the vibrion-septique group as defined on page 389, b. They are by no means all of the same species, but show on careful study various differential characters. A definition of these characters is forthcoming. If these strains are to be accepted as the etiologic agents in cases of bradsot, then we must say that bradsot is not a disease sui generis.

I realize fully that my lack of case histories must qualify any statement as to the causative rôle that these organisms played in the death of the sheep from which they came. The final criteria to be used in judging of what is braxy and what is not braxy, of what constitutes anaerobic infection and of what must be ruled out as not constituting anaerobic infection are, to my mind, still to be established. But the fundamental bases for such criteria should be etiologic and histopathologic ones.

The fact that pathogenic anaerobes may be found in animals dying of other causes is, to my mind, entirely beside the question. Careful and intelligent determination of the quantity and specific nature of the anaerobic invaders in fresh cadavers of sheep showing "bradsot" lesions and in fresh cadavers of sheep dying of other causes is the only way to make observations that can be considered of great value.

Data concerning other strains isolated will be found in the next part of this article.

A note as to the subdivision of the groups discussed is perhaps in order. Various authors have noted differences in anaerobes which had been classified as one species by other workers. Many of these differentiations depended on culture-contaminations. Under this head I should classify Titze and Weichel's strains, Markoff's proteolytic and nonproteolytic chain-forming strains, and Klose's strains. Some divisions depended on an association and further division of unrelated organisms. Kirsten investigated many carefully-isolated strains of what he calls the edema bacillus. He found the organisms in feces and in the blood of various animals. Most of them were nonpathogenic. Kirsten describes 10 types which can be distinguished by morphology and by cultural characters. I interpret these strains to have been members of various saprophytic and parasitic groups. Some differentiations have been made whose value I am unable to determine. Poels, whose original article is inaccessible to me, cultivated blackleg bacilli from what I take to have been the inoculated thigh of guinea-pigs. He noted differences in the gas formation in the time of sporulation, and in the pathogenicity of his strains. Grosso divides his blackleg strains into "Rauschbrand"—nonpathogenic for mice—and "Pseudo-rauschbrand"—pathogenic for mice. Robertson (Med. Res. Comm. 39, 53) has divided vibrion septique into 3 groups on the basis of the agglutination reaction. Weinberg and Séguin (b) note minor differences in their vibrion-septique strains.

A few details as to the differences between the strains in my collection must be included. Most of the blackleg strains behave alike, so far as they have been studied. These I call temporarily "typical" blackleg. They form heavy citron-shaped orgonts, are quite highly pathogenic for guinea-pigs and are not pathogenic for rabbits. Their colonies are similar. A few other strains behave differently. One is different in morphology and is only slightly pathogenic for guinea-pigs. Another has never been observed to form citron-shaped orgonts, another forms colonies that are unique. A careful investigation of other characters of these organisms is in progress. Until this investigation is finished I must decline to divide the group in a dogmatic manner. The strains of the vibrion-septique group are divisible into subgroups by agglutination, by colony formation, and to some extent by morphology. I must also decline to arrange these divisions for the present.

III. The Anaerobic Infections of Animals as Recorded in the Literature: A Critical Analysis of the Anaerobic Infections of Cattle

(*a*) *The History of Cattle Infections Up to* 1905.—This article deals with the organisms involved in the anaerobic infections of animals. I am in no way prepared to discuss with authority the subject of the pathology of the disease of blackleg in cattle, and must be content to refer the reader to some of the notable works on that subject. It will be convenient to discuss simultaneously the two types of organisms and the diseases caused by them.

Anthrax and symptomatic anthrax (blackleg) are two diseases that are in general so similar that ancient and medieval authors do not distinguish them. According to Arloing, Cornevin, and Thomas, who have most carefully reviewed the literature from ancient times on, a satisfactory and logical classification of anthracoid diseases was not made till Chabert undertook to arrange them according to clinical symptoms in 1782. He classified them thus:

Fievre charbonneuse: "When the disease develops without manifesting its existence by external tumors, it is anthrax fever or internal anthrax."

When tumors appear, it is "essentiel" or "symptomatique."

Charbon essentiel: "It is "essentiel," when the tumor begins at the onset of the disease without former symptoms and without signs of sickness except those which result from its existence, the tumor being at first small, hard, renitent, painful; then enlarging, and only after enlarging producing grave constitutional symptoms. When incised these tissues are black, like gangrenous tissues."

Charbon symptomatique: "It is symptomatic, when the tumor follows a rise of temperature, loss of appetite, arrest of digestion, chills, and rigidity."

Feser is given credit for being the first to distinguish Rauschbrand from anthrax in recent times (1876).

Bollinger was the next to publish material on the subject (1878).

Arloing, Cornevin, and Thomas, in 1879 and following years published a series of papers concerning the disease, which afterward were condensed in book form under the title "le charbon symptomatique du boeuf" and appeared in two editions, the latter in 1887. They reviewed all the literature available on the subject and published accounts of their own valuable researches.

(*b*) *Vibrion Septique and Atypical Blackleg Group Infections of Cattle.*—Malignant edema has often been reported in cattle. I have neglected to collate as long a list of such reports as I might have done, and I am ignorant of the period in which such infections were first recognized.

Puerperal blackleg has often been discussed. Dr. Meyer informs me that some cases of so-called puerperal blackleg are not due to anaerobic invasion. Many cases have, however, been reported as due to organisms of the vibrion septique group.

Some reports of unusual blackleg infections cannot be accurately placed.

As stated before, I am in no position to express an opinion on the pathology of these diseases in cattle. I think that certain suggestions are in harmony with those of Foth and others, and that on the analogy of the behavior of these diseases in other animals certain lesions will be found characteristic in cattle. Blackleg organisms show in general a preference for skeletal and heart muscle, and they produce a greater amount of hemolysis and a smaller quantity of edema, gas, and transudate than do those of the vibrion septique group. Gas is probably most frequently in the form of minute bubbles in the muscles themselves. The organisms of the vibrion septique group grow well enough in muscle but will probably be found to kill, in nature, most often before muscle invasion is extensive. They prefer areolar tissue and mucous membranes and serous linings to muscle. Muscle lesions when present probably do not show the dry, spongy, almost brittle character of blackleg muscle lesions and may be markedly infiltrated. The organisms of the vibrion septique group produce abundant gas in

areolar tissue and in muscle, where it is in larger bubbles than in blackleg lesions, and where it is interstitial in its distribution, and they produce little or no gas in the mucosa. The type of infection, apparently rare, in which the mucosa of the alimentary canal are markedly injected may well be termed "braxy" or "bradsot" of cattle.

This should be emphasized: When a worker with suspected blackleg material from a cow finds both true blackleg and an organism of the vibrion septique group present in the affected muscle of that cow, he has no right to conclude that the organism of the blackleg group alone was the cause of death of the cow; and a worker who finds an organism of the vibrion septique group and no organism of the blackleg group in the affected muscle of a cow dead of a blackleg-like disease is as justified in pronouncing such a strain of the vibrion septique group to be the cause of the death of the cow as he would be justified in pronouncing the disease "blackleg," were the organism of the blackleg group. That organisms of the vibrion septique group are pathogenic for cattle needs today no further proof. Why then persist in overlooking such infections?

(*c*) *An Oedematiens Group Infection in a Cow.*—Kerry describes an organism of oedematiens type which he isolated from a cow that was thought to have died of blackleg. The organism, according to its description, resembles the B. oedematis maligni II, described by Novy in 1894 and isolated by him from a guinea-pig that had been inoculated with milk nuclein. Kerry did not work with Novy, as has often been stated. Whether exact cultural comparison of the two strains was ever made I am unable to determine. Kruse identifies the two strains and names them B. oedematis thermophilus Novy. Migula renamed the species B. Novyi, which name is probably the least likely to lead to confusion. As identifications by means of printed descriptions are unsound, we must let the specific affinities of Kerry's organism go undefined until a strain can be compared with that of Novy and with those of Weinberg. Kerry's organism is far more pathogenic for guinea-pigs than any of the strains of B. Novyi in my possession, and the lesions that it produces (Kitt, e) resemble more closely those produced by the oedematiens strain Joly.

(d) The Epidemiology of Anaerobic Cattle Infections.—Under this above title must be considered the contributory mechanic-traumatic factors leading to infection.

Blackleg as a Wound Infection: Most older authorities agree that blackleg is a wound infection, although frequently the wound may not be large enough to be demonstrated. There are many accounts of necropsies in which no external lesion was found. Therefore many authors have concluded that infection probably often occurs through the alimentary canal.

Blackleg as an Alimentary Canal Infection: The idea permeates the literature that malignant edema is a wound infection due to much graver wounds than those to which blackleg is due. Textbooks all give the disease as a wound infection, never mentioning possible infection through the alimentary canal, though it is now recognized that braxy may infect sheep in this manner, at least occasionally (see p. 431).

I conceive the broad epidemiologic factors in these diseases to be as follows: In the soil in general, wherever life processes occur to any appreciable extent, organisms of the anaerobic group are to be found. The vast majority of these organisms are probably not truly parasitic at all. Different species possess different powers of tissue penetration. To some extent different animals possess different defensive powers against the various species of anaerobes. In general the most highly pathogenic species of anaerobes are comparatively rare in their distribution and wounds do not become heavily contaminated by them and do not frequently become infected. But in regions where animals have died of anaerobic infection since time immemorial there occurs such heavy contamination of wounds by anaerobic organisms that such wounds frequently become infected, and animals ingest quantities of pathogenic organisms, and the chances for infection are multiplied, and whatever be the factors promoting infection, animals occasionally develop the disease, and the district becomes known as a "Rauschbranddistrikt."

That valleys and meadows and swamps should be the most dangerous places is easily understood, when one considers that they are the most closely grazed, that valleys are natural highroads, and that mud is a very active agent in the promotion of wound infections. Then, too, it must be remembered that the disease is one of summer time, in other words, of the grazing country. Doubtless farmers are more careful to destroy carcasses of animals dying near the stables.

To what extent excessive infection of the soil is due to other factors than the contamination of the soil by dead animals, probably no one is at present prepared to state. Le Clainche and Vallée showed that blackleg organisms were present in the feces of cattle which inhabited "blackleg districts." The carelessness of stockmen in the disposal of carcasses is certainly a factor in the spread of these diseases: District veterinary Kjoss-Hanssen of Egersund, Norway, states that as soon as sheep are known to have bradsot the peasants slaughter them, the bodies are butchered, the intestines are thrown to the wind and weather or at most buried in mounds, wherefore contagion is spread over the fields as in the old days. Hamilton, (a, 292) speaking of the Scotch shepherds, says that if the body of a braxy sheep is so decomposed that it is unfit for food it is left in the fields after skinning, or may be thrown on the manure heap. Marsh speaks of a farmer in Wyoming who let 20 carcasses of sheep, dead of blackleg, lie long in a field. Wulff (673) says: "Bis vor wenigen Jahren wurde diese Krankheit (Geburtsrauschbrand) allgemein dem echten Rauschbrande

zugezählt, und noch heute vertritt mancher Praktiker (Honecker) den Standpunkt, dass diejenigen Fälle, die nicht als puerperales malignes Oedem positiv erkannt werden, als Rauschbrandverdachtsfälle wie Rauschbrand veterinarpolizeilich zu behandeln und zu entschädigen sind." It appears to me that Wulff is entirely wrong in implying that there is no need of destroying bodies of animals dead of malignant edema infections. His point of view is directly traceable to the oft quoted idea that the bacillus of malignant edema is universal in its distribution and contaminates every open wound. This in turn is due to the contamination of cultures of nonproteolytic pathogens with the universal proteolytic forms. I should suggest that it were a greater crime against society to leave unburied or undestroyed a cadaver dead of a vibrion septique infection than one dead of a blackleg infection—the organisms of the vibrion septique group have a far greater range of possible hosts among domestic animals, and they are probably, in many cases, at least, quite as pathogenic for cattle as are blackleg organisms. Such laws as exist concerning the destruction of blackleg carcasses should most certainly be construed to cover carcasses of animals dead of both types of infection.

It is conceivable that one type of anaerobe may be responsible for most of the infections of a given district. Such a point should, however, never be taken for granted in any one case. It is also conceivable that one type—perhaps the blackleg type—may infect through the alimentary canal or by some unknown means with more facility than do the organisms of the other type.

It is evident from this discussion that the mode of infection in blackleg, beyond its being an occasional contamination of gross wounds, is entirely unknown. Most discussions as to the portal of entry of the blackleg bacillus revolve around wound infection of some sort, external or alimentary. Because of the fact that blackleg organisms do set up local processes where they are inoculated, and because in braxy the site of inoculation and the site of maximum proliferation is apparently in the alimentary canal, it seems highly improbable that blackleg is frequently a primary alimentary canal infection. It appears to me that the possibility of insect transmission of blackleg has been seriously neglected.

Insect Transmission of Blackleg and Allied Diseases: I have discovered no reference in any textbook to the rôle that may possibly be played by insects in blackleg infection. The idea of such a mode of infection is, however, an old one.

Hintermeyer describes an epidemic of quarter-evil, which raged in the summer and autumn of 1846 among the deer of the park of Duttstein, which Budd quotes somewhat inaccurately: "Als wesentlich habe iche hier noch zu bemerken, dass die Bremsen, und zwar (a) die grosse Rind-bremse (Tabanus bovinus), (b) die Regen-bremse (Tabanus pluvialis), und (c) die Blind-fliege (Tabanus coecutiens) welche im verflossene Sommer in unzähliger Menge vorhanden waren, wohl mit Recht als die theilweisen Träger des Contagiums anzusehen sind, und daher einere grössere Ausdehnung der Seuche verursachten. Diese setzten sich gewöhnlich zu Tausenden, auf die Cadaver der gefallenen Thiere, saugten die aus Maul, Nase, und After kommenden Profluvien ein, verliessen sodann die Leichen, begaben sich sofort auf gesunde Stücke stachen ihren von Contagium Saugrüssel in die Oberfläche der Haut ein, und inoculierten auf solche Weise das Seuchengift." We cannot be sure, of course, of the identity of this epidemic.

Guillebeau suggests that the larvae of bot flies (Hypoderma bovis) may cause infection of cattle in their wanderings from the alimentary canal to the skeletal muscle and to the skin; they make minute canals through the flesh. I

have found no confirmation of this suggestion in necropsy reports. Eggimann states that there is no Rauschbrand in Emmentahl and there are few warbles in hides from there, while skins of cattle from other valleys contain warbles.

I have found only one reference to experimental work in insect transmission of blackleg. Sauer caught common flies and biting flies which had passed a Rauschbrand cadaver. A small number of heads of these animals sufficed to produce typical Rauschbrand in guinea-pigs when inoculated subcutaneously. The bacilli were shown to multiply in the bodies of the flies, but the newer generation were smaller and weaker. Flies which had been on Rauschbrand cadavers were placed with wounded guinea-pigs, and they infected them in two cases. It was not shown whether the bites of flies could infect guinea-pigs.

A Laboratory Infection by an Organism of the Vibrion Septique Group.—I think the possibility of anaerobic infection by a small number of organisms entering through a minute wound, as would be the case in insect inoculation, has been overlooked. In this connection I may state that I recently infected my forefinger at the distal segment with a few washed organisms of a 24-hour culture of strain AS, of the vibrion septique group. The wound was a minute prick with a fine capillary pipet, and it was not more than 2 mm. deep. The finger became painful in 5 hours and swelled slightly, but was not feverish nor did it become cooler than the others. It was carefully kept warm to foster an active circulation. Twenty-four hours after the inoculation, however, on account of neglect, the finger became chilled and it immediately swelled perceptibly. Two hours later it was immersed in hot water and the swelling went down somewhat. Pain and swelling remained in the distal segment of the finger for a month, though fever and a tendency to suppuration were not noticeable. Apparently the infection lingered in the synovial membranes or in the tendon sheath. This is a case of invasion by an organism of the vibrion septique group in which no necrotic tissue was present, in which the size of the wound was infinitesimal, in which the depth of the wound was slight, in which no foreign débris was introduced, and in which no appreciable amount of culture toxin accompanied the organisms. But it will be noted that the organisms introduced were in the vegetative state and had been actively multiplying shortly before they were inoculated. I am confident that had circulation for some reason been partly suspended in the infected finger, the results would have been serious. I have found no other record of a laboratory infection by anaerobic invaders.

Perhaps it will not be considered mal apropos if I also introduce a few references as to transmission of anthrax by insects.

Virchow says: "Most commonly insects with piercing probosces effect the inoculation, such as gadflies (bremse); but flies which make no wound may also implant the poison on the skin by their soiled wings and feet."

Bourgeois expresses himself to the same effect.

Budd (1863) gives various points indicating insect transmission of anthrax. All but one of the cases of malignant pustule of the face which he has seen occurred in hot weather, in summer and autumn, while malignant pustule of the hands occurs all the year around. He cites two cases of women who were bitten by flies and developed malignant pustule at the site of the puncture, and another case which was caused by the puncture of a gadfly that came out of a fleece of wool.

Raimbert concludes (1869) from certain experiments that anthrax may be transmitted by flies, and that it is more probable that it is transmitted by flies that feed from cadavers than from flies that live by bloodsucking only.

Koch states that practically all the anthrax of the Russian steppes occurring at harvest time is due to insects, but does not give proof of his statement.

Minzmain (1914) successfully infected guinea-pigs with anthrax by means of the biting flies Stomoxys calcitrans and Tabanus striatus. The flies were applied to the hosts shortly before the death of the latter, and immediately after or 10 minutes later were applied to healthy guinea-pigs. The exposed animals died during the evening of the third day of typical anthrax.

Morris infected wounded guinea-pigs with anthrax by means of nonbiting flies.

As far as I can make out there is a generally accepted opinion among entomologists and veterinary scientists that anthrax may be, and frequently is, transmitted by flies.

The nature of blackleg is not so different from that of anthrax. The anaerobic organisms in the vegetative form are easily killed by air, as I have found in using the Barber technic of isolation. It is possible that inoculation of spores even in small numbers may prove infectious, but it is my opinion that inoculation of vegetative rods is much more so. If the statements of Sauer are correct, we may conclude that vegetative forms of the bacillus may be found in the bodies of flies. It is my opinion that insect transmission of blackleg is exceedingly likely. When one sees the charts representing the incidence of blackleg in "Le charbon symptomatique du boeuf"; how the malady increases in May and reaches its zenith in July, decreasing and practically disappearing in some years in September and in others in October, one is strongly inclined to look no further for a means of transmission of the disease. But it must be borne in mind that summer is the time of abundant grass in Switzerland. In California, Dr. Traum assures me, blackleg is to be expected whenever the grass is abundant, in fall or spring as the case may be. In Switzerland (Arloing, Cornevin, and Thomas) anthrax and blackleg occur at the same time; in California anthrax occurs in summer and fall when the ground is bare. It has frequently been noted that the fattest cattle die of blackleg, the finest sheep die of braxy. The explanation has been sought in the greater abundance of sugar in the muscles of animals that are well conditioned. Möller states that guinea-pigs that are sugar fed show much gas on blackleg inoculation, those that are not sugar fed show less, those that are starved show none at all. Guinea-pigs that are starved are less likely to succumb to blackleg than those that are well fed. I should say that this element must be thought of as a predisposing factor and not as a factor leading to infection.

Farmers in some portions of California often attribute blackleg infection to the drinking of contaminated water. Farmers of other districts in which the water is excellent are emphatic in asserting that the infection is in the food. There is not necessarily a single avenue of infection in these diseases, no more than there is in anthrax, and the demonstration of one mode of infection does not prove that it is the only or usual mode.

One strong point opposing the view that vibrion-septique infections are not rare in cattle is the apparently comparatively satisfactory protection furnished cattle by vaccination. Kitt (a) states that in Bavaria between the years 1898 and 1907, 0.144-0.320% of vaccinated cattle died of blackleg and 0.551-2.497% of unvaccinated cattle died of blackleg. Balavoine's figures for Switzerland are not very different from Kitt's. Blackleg aggressin is said to protect cattle even better than the bacterial vaccines. To what extent vibrion septique strains have been unintentionally used for vaccination purposes it is at present impossible to estimate.

The epidemiology of these infections is one of the most interesting in human and animal pathology. I regard it as an almost virgin field for investigation. It should be studied, and studied exhaustively, not by the veterinary pathologist, but by the trained anaerobist, who will investigate thoroughly every case that comes under his observation, and will not be satisfied that the first organism that comes to hand is the only one involved in the death of the animal before him, until he has looked carefully for others. Under the investigation of the epidemiology of blackleg and malignant edema should come the investigation of the immunity of older animals to these diseases, the immunity of animals from districts free of blackleg and braxy, the relative immunity of cattle and sheep of different breeds, infection of wounds and infection through the alimentary canal, infection by means of insects, and infection through causes that are now unknown to us—innumerable problems could be suggested. We shall not, however, be in a position to make such problems a profitable study until more is known as to the specific members of the groups involved.

(e) Conclusions as to the Anaerobic Infections of Cattle.—The conclusions as to the anaerobic infections of cattle which I derive from study of the above mentioned material and from a study of the literature of the subject of animal infections are:

Theoretical considerations lead one to conclude that cattle infection by a single species of anaerobe may take place, and that this species may be an organism of the vibrion septique group, of the blackleg group, or of the oedematiens group; or a mixed infection may take place by members of the different groups or by two members of the same group, or some other combination of organisms may invade the tissue. I find no record of B. Welchii infection in cattle. There is probably no reason why it may not be found as a wound infection, nor why other organisms may not be found, nor why proteolytic organisms may not also occasionally invade bovine tissue with the non-proteolytic invaders as they are known to do in man and small animals. Probably some of the ideas concerning the proteolytic nature of malignant edema are due to the observation of such cases.

Practical considerations lead to the following conclusions: Cattle are frequently subject to spontaneous infection by members of two groups of anaerobic invaders, the blackleg group and the vibrion-septique group. Both types of these infections are, in the vast majority of cases, diagnosed as "blackleg," "Rauschbrand," or "symptomatic anthrax." Infection by members of the first group is commoner than that by members of the second group, but infection by members of the second group is by no means to be ignored and probably should be considered in cattle immunization.

It is possible that a member of one group may be the predominant invader in one district, and a member of the other in another, which indicates the necessity of determining the bacterial agents involved in "blackleg" infection in various districts.

It is also possible and, to my mind, probable, that factors exist that induce anaerobic infection in cattle, whose identity it is important to solve. Beyond the knowledge that these infections may follow wound contamination we know nothing positive as to the mechanism of anaerobic infection in cattle.

Careful, intelligent, and thorough bacteriologic investigation of fresh cadavers of many animals dying of spontaneous "blackleg" infection is much needed, and connected with it is the need of an exhaustive epidemiologic survey of the field. The attitude of the veterinary profession has been that we already possess all the necessary knowledge concerning blackleg. It appears to me that we are in the morning of the investigation of blackleg and allied diseases, and that what we today know is but an inkling of the true state of affairs. An investigation of the subject that will properly correlate and solve the problems involved can be undertaken only after many workers have made studies of many cases from different districts. The reasons why this has not already been done are two: anaerobic technic has not been adequate for such study, and the idea of the monospecificity of these invasions has tenaciously held the field.

(f) The Immunization of Cattle.—It is not my purpose here to enter into a discussion of the extensive subject of the immunization of cattle against blackleg by bacterial products.

Arloing, Cornevin, and Thomas were the pioneers in immunization by killed and attenuated virus. Kitt modified their technic and also introduced serum virus inoculation for the production of immediate protection. His article in Kolle and Wassermann's Handbuch (IV, 1913) may be referred to as a modern authority on the subject. The use of filtrates for immunization of cattle was first suggested by Roux in 1888 (b) on the basis of protection afforded to laboratory animals by filtrates of infected guinea-pig tissue. Today various means of immunization are in use. Injection of powdered attenuated muscle virus and of pellet vaccines is still widely practiced, and tissue and culture filtrates, the so-called "aggressins," are apparently gaining in favor because of the safety attendant on their inoculation. Serum immunization is used for herds in which blackleg has recently occurred.

Suffice it to say that immunization of cattle against blackleg is regarded as highly necessary in some districts and as advisable in others. But it must be emphasized that such immunization is not always successful and that inoculation infection ("Impfrauschbrand") is not uncommon. These facts fit in so plausibly with my own figures and with the thesis of this paper that I am forced to conclude that the subject of the immunization of cattle deserves a careful investigation.

The only case of inoculation blackleg that has come to my attention is that of Dr. Haslam (private communication). The causative organism was a member of the vibrion septique group of the general type of the strain called AS. The spores sometimes lie sidewise in the bacillus and this type of organism is one of the most highly invasive and highly pathogenic of the group, and grows unusually actively on meat medium. The calf from which this strain was derived was one of several killed by a pellet vaccine. It is conceivable that such an organism as this would be insufficiently attenuated by the conventional procedure, which satisfactorily attenuates organisms of the blackleg group. Careful observation of details of this sort will perhaps clear up most cases of inoculation blackleg.

Animals immunized against the vibrion septique group are not immunized against blackleg, and vice versa. All immune reactions separate the two groups. So far as I am able to discover by a careful search of the literature, there is no sign of failure on the part of one strain of a group to immunize against other strains of the same group.

Robertson (b) found that the four types of vibrion septique which she distinguished on the basis of the agglutination reaction, all produced a toxin which was neutralized by a monovalent antitoxin.

It is my present opinion that cattle should be immunized against both groups, but perhaps such practice should depend on the nature of the organisms found to infest a particular locality. Vaccine for the vibrion septique type should be made separately from blackleg vaccine and probably should be more severely attenuated. It must be emphasized that the worker in charge of the manufacturing of such vaccine should be thoroughly familiar with both types of organisms and should check his cultures continually.

2. THE ANAEROBIC INFECTIONS OF SHEEP

Sheep infections may be considered under four heads: (1) blackleg; (2) malignant edema or vibrion septique infections; (3) braxy or bradsot, and (4) black disease.

(*a*) *Introduction.*—Distinction between these infections is extremely difficult, if we are to judge from the accounts in the literature. One is inclined to make the statement that the nature of the diagnosis depends more on who describes the disease than on what it is. One point to be brought out is that clinical characters have been so emphasized in connection with the different sheep infections that etiologic factors have been largely lost sight of. Another point for consideration is that gangrenous infections of sheep are perhaps different from gangrenous infections of cattle; that is, the specific nature of the host must be considered in diagnosis, as well as the specific nature of the parasite. A possible difference between infections of cattle and infections of sheep appears to be the greater selective preference of the organisms for mucosa of the fourth stomach and the intestines of the sheep. It may well be, however, that closer study will show that this difference is one referable solely to the specific invaders or to the position and nature of the portal of entry of the infection.

Another difference that may exist between sheep and other animals has probably been the cause of much confusion as to sheep infections. This is the phenomenon of the rapid decomposition of sheep cadavers. While cadavers of cattle dead of spontaneous anaerobic infection and those of experimental

animals dead of nonproteolytic anaerobic infection show a marked slowness in decomposition, those of sheep are so frequently referred to as readily decomposing that one must conclude that the nature of the sheep in this regard is different from that of other animals. Krabbe, in 1875, mentions the fact that sheep dead of bradsot decay so rapidly that dogs and ravens will not eat them. On the other hand, we frequentlly hear that the flesh of cattle dead of blackleg may be eaten by man. Jensen, Hamilton, and Gilruth all mention the rapid decomposition of cadavers of sheep dead of braxy. Gilruth (b, 572) goes into some detail in the matter.

(*b*) *Blackleg in Sheep.*—It is evident that many diagnoses have been made of blackleg in sheep when the diagnostician did not know the difference between blackleg infections and malignant edema or vibrion septique infections in sheep. This is, of course, natural, as many diagnosticians do not know the difference between blackleg in cattle and malignant edema or vibrion-septique infections in cattle. Textbooks frequently mention blackleg in sheep, but the actual notes of the occurrence of this disease are rare.

Hadleigh Marsh reports an outbreak of blackleg in sheep. His case is that of a flock of rams into which a ewe was introduced by mistake. The rams fell to fighting and many of them died of a disease which he identified as blackleg.

The invasive organism isolated was typical blackleg (strain RM).

Strain RG, which I isolated from a sheep thought to have died of bradsot on the estate of Graf Bülow in Halle (a sample of the muscle of which animal was sent me by Dr. Jensen), is a blackleg group strain. It is a species distinct from any other in my possession and fulfils all requirements as to blackleg characters as stated on page 388, a.

Various authors report experimental infection of goats with blackleg, but I know of no accurate report of natural infection of goats with this disease.

(*c*) *Malignant Edema and Vibrion Septique Infections of Sheep.*—Records of these infections show that there is a group which must be classified as wound infections.

(*d*) *Braxy or Bradsot.*—The best account of this disease that I have found is that given in the Reports of the Journal of Comparative Pathology and Therapeutics (1902, 15, p. 181). I presume that this review is to be attributed to one of the editors of the journal, Dr. J. M'Fadyean or Dr. Stockman. I quote rather liberally from this paper because some of the sources are inaccessible to me. It is primarily a review of a paper by Professor Hamilton of Aberdeen University, to whom the reviewer refers as the "author." Hamilton

finally identifies the braxy of Scotland with the bradsot of Norway, Denmark, Iceland and Germany. There have been many papers on the disease which lack satisfactory evidence as to its nature (182). "The first really scientific account of the pathology of the disease is that by the Norwegian Government veterinary officer, Ivar Nielsen, who likewise must be regarded as having discovered the micro-organism which is its cause. Previous to the publication of Nielsen's paper in 1888, and even for some time afterwards, the malady was considered to be anthrax."

". . . braxy is certainly not anthrax. . . . There is another disease, however, with which it has even closer points of resemblance, namely, quarter evil (Rauschbrand). Quarter evil is said to be unknown also in Iceland, and the two diseases, and the organisms causing them, when compared, are readily enough distinguishable. Thus braxy is never accompanied by any external lesion, as is the case in quarter evil; it also runs a much more rapid course than that disease.

"Braxy is, therefore, neither an anthracoid disease nor is it to be confounded with any other malady; it is a disease *sui generis*."

(p. 183) "Nielsen has defined it as a gastromycosis, excited by a specific bacillus introduced with the food into the fourth or true stomach, and believes that the disease may either pass into a general affection, or may occasion the death of the animal through absorption of bacterial toxins formed by the organism germinating locally in a part. In these views he was supported by Jensen, who concluded as a result of the researches of previous investigators that bradsot is an acute or even extremely acute, infectious disease which begins as a hemorrhagic inflammation of the mucosa of the fourth stomach, which is accompanied by the formation of gas in the alimentary canal, especially in the stomach, and which kills the animal in some cases by a general infection, in others apparently by toxic poisoning, or possibly in others still by dyspnea caused by tympanites."

(p. 284) "*Age of Animals Attacked.*—Accounts of the disease from all countries seem to uphold the allegation that the first year's animals are far more liable to braxy than those more mature."

Postmortem Appearances.—According to Jensen's description, the following are the chief postmortem appearances:

When the animal is killed during an attack the essential change is a dark bluish-red, somewhat swollen patch on the fourth stomach. This patch increases in size, so that toward the end of the attack a great part or the whole of the stomach may be hemorrhagic or serous hemorrhagic. The fourth stomach and adjacent part of the bowel are devoid of food, but sometimes contain a bloody fluid. The hemorrhagic infiltration may spread from the fourth over the other stomachs, partly over the duodenum, or even over a great portion of the intestine, while other parts of the intestine may be hemorrhagically injected. In the cavities of the body there may be a little serous fluid. The blood is dark, and may be clotted; and the spleen is occasionally somewhat swollen, but may be quite normal. The liver is pale, brittle, and degenerated, and in extreme cases the friability is probably from postmortem causes. The kidneys may be normal or somewhat degenerated; not uncommonly, however, they are enlarged and very brittle or even diffluent. The cadaver decomposes soon, and before long the hind quarters become blown

up with gas, the verge of the anus protuberant, the skin takes on here and there a bluish color, and the wool is easily detached; sometimes the skin bursts and a serous hemorrhagic fluid is seen oozing from the subcutaneous areolar tissue.

The lesions in typical cases are summarized by Hamilton:

"1. The absence of any external manifestation of the disease as in the case of the slough of quarter evil.

2. The tendency which there is, both during life and after death, to the production of gas.

3. The presence of sero-sanguinolent effusions into the various cavities of the body and into the subcutaneous areolar tissue.

4. The tendency to blood-staining of the tissues.

5. The absence of inflammatory manifestations.

6. The occasional, but by no means invariable, hemorrhagic infiltration of the mucous membrane of the fourth stomach, with, from time to time, ulceration or digestion of the surface of the infiltrated parts.

7. The distention with food of the first three stomachs, and the absence of food in the fourth or true stomach, and usually in the intestine. The presence of a little brownish-red grumous liquid in the fourth stomach.

8. The occurrence, occasionally, of hyperemia of the large vessels in the walls of this organ, accompanied by blood-staining of the surrounding tissues.

9. The absence of extensive hemorrhage into the musculature of the body; the absence of gas-production in the muscles.

10. The invariable presence of the braxy bacillus in the liquids, tissues, and organs of the body."

"The organism of braxy is apparently very closely related to that of quarter evil and of malignant edema. The three form a group which stand isolated, and which have intimate mutual relationships. In fact, when growing in solid media there is much difficulty in distinguishing them."

Fröhner described a bradsot-like disease in sheep. He observed the symptoms closely: the animals became depressed, had difficulty in breathing, their abdomens swelled, they were in terrible pain, and they ground their teeth and were restless. They died in a few hours. Decay appeared soon after death. The subcutaneous connective tissue of the head and neck and of the side of the rump showed a gelatinous red infiltration, the muscles of the neck and the back and the cutaneous muscles of the belly were those most affected. The mucous membrane of the nasal cavity and of the turbinates was deep red and swollen, the lymph glands were swollen and infiltrated, the heart muscles grayish red with brownish red spots, the pericardium contained much red watery fluid, the pleura of the ribs were a diffuse deep red and there were also spleen, kidney, and liver lesions. It is possible that these infections took place by way of the nasopharyngeal mucosa, as did the hog cases described by Meyer, as lesions in the head region are not mentioned in necropsies of animals dying of the form of the disease which affects the abomasum, and it is also possible that infection was caused by the bites of flies that could reach only the head of the sheep.

Gilruth (b and c) identifies certain sheep diseases of Victoria and Tasmania and New Zealand with the braxy of Scotland and the bradsot of Scandinavia, and reports an investigation of the diseases of Tasmania and of

Victoria. He makes, however, the mistake of presupposing that the etiologic bacterial agent of a given outbreak is the same in all cases, and therefore he studied carefully only one strain from Tasmania and one strain from Victoria.

Gilruth makes some interesting epidemiologic observations. He connects outbreaks of braxy with dryness and bareness of the soil and with forced ingestion of soil. In common with others he notes that the animals affected are young and in excellent condition. He produces a relative immunity to inoculation by feeding culture to sheep, and holds that this fact disproves, in the present state of our knowledge, the possibility of natural infection by the digestive tract. I cannot agree with this latter statement. Gilruth failed to produce braxy by feeding cultures in a gelatin bolus which contained glass fragments.

Miessner (quoted from Jensen, below) published a paper on the etiology of bradsot in Germany. According to him the lesions produced by bradsot in Germany are not as uniform as in the North, the mucosa of the fourth stomach are less inflamed or not inflamed at all, and bacilli are not always present. His conclusions were that the so-called bradsot bacillus was not the cause of the disease and that the etiology of bradsot was entirely unknown.

Titze and Weichel report extensive experiments with bradsot. Their history of the subject is detailed. They agree with their predecessors that the Scandinavian bradsot and the Scotch braxy and louping-ill closely correspond, but state that no common etiologic factor has been demonstrated for them. (Louping ill was probably wrongly associated with braxy by Hamilton.) They then discuss all reports concerning bradsot in Germany and come to the conclusion that in Germany the disease presents the same characteristics as in the other countries, including the predominance of the disease in winter, but that most of the German cases occur when the sheep are shut up instead of when they are put out to pasture. Moreover, animals of all ages sicken in Germany. The occurrence of the disease is sporadic.

They conclude that the etiology of bradsot is entirely obscure, that the bradsot bacilli are mere cadaver bacilli, and that the diagnosis of bradsot rests on clinical and pathologic characters.

Jensen (c) energetically attacked Miessner, and Titze and Weichel, showing that they may well have considered as bradsot cases that were not bradsot, that Miesner falsely based his accusations that Jensen and Nielsen had worked with old cadavers; also citing protections afforded by vaccination of sheep with bradsot vaccine.

Jensen mentions in this account that of 1,545 sheep that were fed a prophylactic dose of pure culture of the organism, 5 died of typical bradsot; adding that such cases have also been reported from Iceland.

He gives careful descriptions of stomach linings of sheep that died of bradsot in Iceland; these stomachs had been removed immediately after the death of the animal and placed in formaldehyd. Jensen insists that infection takes place in various foci in the mucosa that are hemorrhagic and necrotic; that the bacilli penetrate at these spots and enter the loose connective tissue of the submucosa, there multiply extensively, and then invade the whole mucosa. His illustrations seem adequately to substantiate his statements.

I fully agree with Nielsen in his interpretation of the effect of cold on these infections. We know from many records of human pathology during the war (Taylor, Hartley) that any influence tending to decrease the circulation in a part allows in that part the development, even in the absence of necrotized tissue, of a gas gangrene which would never have occurred had

circulation been plentiful. The blood, with its leukocytes, is in these diseases the greatest bodily defense. It is most likely, indeed, that chilling of an animal will permit a multiplication of toxic invaders which would never have occurred under normal circumstances. The factor may be looked on simply as a dilution in health, or a concentration, in the case of poor circulation, of the toxin surrounding the first invaders.

Jensen and Nielsen believe bradsot to have its portal of entry primarily in the fourth stomach. Hamilton believes they overemphasize this conception. Nielsen's statement as to the shearing of sheep in the fall in Norway is suggestive of wound infection. It is, however, more than probable that alimentary canal infections may take place. Many infections are mentioned in which no external wound is to be found. I think that the possible minuteness of the necessary portal of entry for these organisms, whether it be external or in the alimentary canal, has been overlooked (see p. 423).

(*e*) *Black Disease.*—In New South Wales a disease of sheep exists which has been thought by some to be an anaerobic invasion. Dodd reports experimental work on this "black disease."

Black disease is very erratic in making its appearance; it is enzootic, occurring in the southern highlands of New South Wales. It appears in late summer (February) and ceases in early winter (May), usually, but not always vanishing with the disappearance of frost. Inland it is a winter disease; the winters inland are warmer than they are farther south. Animals of all ages and of both sexes are affected; the disease is apparently peculiar to sheep; the mortality is variable and sometimes heavy. Stricken animals show few symptoms, they lag behind the others, and die quickly. Necropsies of animals dying of natural infection show thin bloody liquid in the abdominal and pleural cavities and some congestion of the abomasum, occasional injection of the intestine, and injection of the skin and subcutaneous connective tissue. Decomposition appears early. Heart blood of animals found recently dead of the disease, when inoculated into normal sheep, produces local gelatinous edema, thin blood-tinged serous infiltration of the subcutaneous connective tissue, and occasionally gas. Some muscles show a dark hemorrhagic appearance not unlike that found in blackleg cases, and they have a distinct peculiar but not putrefactive odor. Other affected muscles have a clay colored appearance and on incision have a distinctly putrefactive odor. The abomasum is usually congested, and the intestines are occasionally injected, sometimes markedly so. Feeding experiments were negative. Tissue inoculations and culture inoculations sometimes killed and sometimes failed to kill guinea-pigs and rabbits, being more highly pathogenic for the former.

The work mentioned above was done in 1914 with material from animals found dead of black disease and thought to have died only a comparatively short time before necropsy examinations were made. Later research showed that it was difficult to produce the disease in animals with material and cultures from sheep that had just died of black disease. Dodd says: "The conclusion to be drawn from the research work narrated is that the bacteria isolated from the blood, exudates, organs, etc., of sheep found dead of black disease, including that of the braxy type, are agonal or postmortem invaders."

To me it is quite conceivable that black disease may be an alimentary canal invasion by a highly toxic vibrion septique type of organism that kills the

sheep by its toxin and becomes septicemic only shortly before death, escaping in such small numbers that inoculation of fresh tissue not directly involved in the primary process will not reproduce the disease in some cases, whereas the inoculation of tissue that has lain for a few hours will do so in all or nearly all cases. Nielsen (Jensen, b) found that the bradsot bacilli do not always become septicemic in sheep.

We have record of cattle infections (Wulff and Laabs, see reprint) that were diagnosed as B. chauvoei invasions, but some of which may have been invasions of the vibrion septique group, which show no muscle lesion at all. We have record of hog invasions by strains of the vibrion septique group, in which no external lesion was to be discovered and muscle lesions were absent, and in which the resemblance to braxy was marked. It is to be expected that material from such cases when inoculated subcutaneously or intramuscularly into the thigh of an animal will produce edema and sometimes gas at the site of inoculation. But Dodd's organisms and Gilruth's organisms and also Titze and Weichel's organisms reproduce, on inoculation, also the injection of the abomasum and of the intestine and other lesions produced by the disease in its natural form. It is to be expected that in the case of artificial subcutaneous or intramuscular inoculation death will occur before the mass infection in the alimentary canal is as great as in the case of internal infections. The injection of the membranes will then also be less than in the case of an alimentary canal invasion. Vibrion septique strains produce strong toxins, and when an infection is under way, it is a matter of only a few hours before death ensues.

Dodd is right in holding a skeptical attitude in studying a disease of this sort; he is to be commended for choosing fresh cadavers for his investigations. He is quite right in saying that the finding of a highly pathogenic anaerobe in the tissues of a dead sheep is no proof that that anaerobe was the cause of the death of the sheep. But the finding of a highly pathogenic anaerobe in the tissues of an animal not long dead demands careful investigation as to its location and quantitative distribution in the body of that animal before one is warranted in stating that the anaerobe was not the cause of the death of the animal. I feel that Dodd entirely underrates the pathogenicity of the organisms of the vibrion septique group, and confounds them with "cadaver bacilli." This is easily understood because of the prevailing confusion on this point. It must be emphasized that the highly pathogenic anaerobes are members of definite restricted groups that are few in number, and that there are a vast number of species of anaerobes that may be called "cadaver bacilli" and have no invasive power of their own. Lumping all anaerobes together as "cadaver bacilli" because most of them are putrefactive is as primitive a procedure as would be the lumping together of all aerobes for a similar reason.

In questioning the anaerobic origin of black disease Dodd is also forced to question the anaerobic etiology of Gilruth's nonwound infection braxy cases. This he has a right to do as Gilruth's observations are not founded on sufficient data to be conclusive. He also questions the anaerobic etiology of the northern braxy. I feel that it would be necessary for him also to question, on the same ground, all anaerobic infections of cattle in which no external lesion is to be found—which, according to Wulff, is true of the vast majority of blackleg cases. The parallelism between the anaerobic cattle and hog infections and these sheep diseases is close.

Grosso identifies the bacillus of bradsot with the malignant-edema bacillus by means of the agglutination reaction.

Zeissler (a) states that the bacillus of Ghon and Sachs is the cause of bradsot in sheep, goats, and hogs, including wild hogs. He and Eugene Fraenkel found the bacillus of Gohn and Sachs in bradsot in a sheep and a goat. I presume that the statement regarding hogs is based on the work of Köves (see reprint). Zeissler asserts that the bacillus of Gohn and Sachs is the one specific cause of bradsot.

(*f*) *The Epidemiology of the Anaerobic Infection of Sheep and Tentative Conclusions as to Such Infections.*—We know that anaerobic invasion of sheep by organisms of the vibrion septique group and of the blackleg group may take place after wound contamination. This phenomenon is entirely analogous to that of cattle infection.

Other infections of sheep, which do not follow the contamination of visible wounds, do not present a patent parallel to cattle infections. The striking differences are two: First, anaerobic infections of sheep are usually caused by organisms of the vibrion septique group and rarely caused by organisms of the blackleg group, and anaerobic infections of cattle are usually caused by organisms of the blackleg group and less frequently by organisms of the vibrion septique group. Second, the infections of sheep are more often localized in the alimentary canal and serous lining of the peritoneal cavity, while in cattle the infective process is more frequently localized in the muscles. Granted that the predominating invaders of cattle, namely, the organisms of the blackleg group, prefer muscle tissue, and the predominating invaders of sheep, the organisms of the vibrion septique group, prefer somewhat the serous membranes, I do not believe that this fact alone explains the great number of alimentary canal localizations in sheep. There must either be a constitutional difference between sheep and cattle in this respect, or there must be a difference in the mode of infection in the two species. It seems to me that a satisfactory explanation of the differences would be covered by two considerations: first, the acceptance of Nielsen's and Jensen's contention that alimentary canal infection is common in sheep; second, the consideration that if insects may transmit these diseases, cattle would be far more exposed to infection of skeletal muscle than sheep, for sheep may be bitten by free flying insects over a very small portion of the body. The fact that bradsot is common in winter and blackleg is common in summer fits in closest harmony with these considerations. Moreover, on inoculation, blackleg is highly pathogenic for sheep, and vibrion septique strains are highly pathogenic for cattle, and a difference in the susceptibility of the two types of organisms for these two types of transmission may explain the discrepancy in the predominance in natural infection of one group in cattle and of the other in sheep.

Data concerning sheep infections are not sufficient to permit a discussion of insect transmission. We may, however, consider more closely the question of alimentary canal invasion. Jensen's evidences as to the mode of invasion in the bradsot of Iceland are probably sufficient, though the reason for such a mode of invasion is unknown. In Germany and in other countries, however, where the lesions are not so uniform, other factors must be considered. The fact that intramuscular inoculation of vibrion septique strains into guinea-pigs and sheep produces gastro-enteritis makes necessary the careful search for a minute portal of entry in bradsot cadavers. This has been pointed out by Hamilton and by Gilruth. The suggestion that organisms of the blackleg group do not show the preference for growth on the abdominal serous membranes that is shown by strains of the vibrion septique group would be worth

considering in the case of suspected bradsot with slight alimentary canal lesions. Vibrion septique strains also vary somewhat among themselves in respect to production of gastro-enteritis in the guinea-pig.

I hope that after the publication of this article it will no more be stated that because bradsot material was capable on experimental inoculation of producing "malignant edema," bradsot cannot be considered to be caused by an anaerobe. No one who has studied the action of anaerobes in the animal body would for a moment hesitate to declare the possibility that bradsot may be an alimentary canal localization of the disease known as "malignant edema." These diseases are analogous to the group caused by one specific organism: B. anthracis. The diseases caused by this organism are known as malignant pustule, wool-sorter's disease, and intestinal anthrax—depending on the site of the multiplication of the organism, and on the mode of transmission and inoculation. Infections of the subcutis and muscles by organisms of the vibrion septique group are called "malignant edema"; infections of the fourth stomach and intestine of sheep by organisms of the vibrion septique group are known as "braxy" or "bradsot"; vibrion septique infections of the lungs and pleural cavity are termed "pneumonia." It is time that more emphasis were placed on the specific etiology of diseases and less on the lesions produced by them.

The chaotic condition of the subject of the anaerobic infections of sheep and the diseases that resemble them is, to my mind, due to several causes. First, although the disease of braxy has long been considered an infection, many of the investigations concerning it have been undertaken from the pathologic-anatomic point of view. Second, when infections have been considered probable, the idea of a specific causative organism has been held to and few cases have been properly studied. Third, the epidemiologic rules applied to infectious diseases in general have been applied also to these diseases, which differ from ordinary infections in that they are caused by accidental invasion by organisms that are frequently present in the alimentary canal of the host organisms to which the host usually shows immunity. Therefore mechanical, physical, and chemical factors must be considered as inducing alimentary canal invasions by anaerobes, and the closest analogues to such invasions are to be found in wound infections and not in contagious infections. Common sense and not dogma should be our guide in seeking the etiologic elements concerned in these diseases, which certainly cannot be included in those infections that must be judged by the four rules of Koch.[11] Feeding experiments have amply proved that these diseases are not transmissible diseases in the ordinary sense. Certain factors to be considered in anaerobic alimentary canal infection are:

1. Gross wound infection in a normal animal. The mucosa being cut by a foreign object or deeply pricked by a sharp point, may become contaminated by a toxin producing organism, and wound infection is far more likely to occur

[11] Koch himself says as to the epidemiologic considerations involved in wound infections (a, 75): ". . . ein vollgültiger Beweis . . . nur dann geschafft werden kann, wenn es gelinkt, die parasitischen Mikroorganismen in allen Fällen der betreffenden Krankheit aufzufinden, sie ferner in solcher Menge und Verteilung nachzuweisen, dass alle Krankheitserscheinungen dadurch ihre Erklärung finden, und schliesslich für jede einzelne Wundinfektionskrankheit einen morphologisch wohl characterisierten Mikroorganismus als Parasiten festzustellen.

"Sollte es denn nun aber möglich sein, diese Bedingungen überhaupt jemals zu erfüllen?"

(100) "Denn je länger ich mich dem Studium der Infektionskrankheiten befasst habe, um so mehr habe ich die Ueberzeugung gewonnen, dass das Generalisieren neuer Tatsachen hier verfrüht ist und dass jede einzelne Infektionskrankheit oder Gruppe nahe verwandter Infektionskrankheiten für sich erforscht werden muss."

than in the case of an infected external wound of the same size for two reasons: the conditions are more anaerobic, and the infective agent probably is usually present in the vegetative form.

2. The infection of a minute wound in a debilitated animal—such a case as that suggested by Nielsen in which freshly shorn sheep are turned out in the cold. Also animals sick from some other cause may come under this head: Kitt's cases of hogs dying of Schweinepest and anaerobic invasion (see reprint) would probably come under this head. Perhaps also Meyer's cases (b) of cattle with liver foci, which were apparently caused by organisms of the vibrion septique group that were nonpathogenic to cattle on artificial inoculation. I believe that abundant cases of this kind might be collated from human necropsies.

3. Infection of wounds either in the alimentary canal or in the liver; these wounds are caused by flukes or other parasites (worms are common in sheep). This possibility has been emphasized by Gilruth.

4. It is quite conceivable that organisms of the toxic type may sometimes find suitable food material so abundant in the alimentary canal that they multiply to such an extent that their toxin overcomes the general resistance of the wall of the alimentary canal and they invade en masse. The "bacterial felt" pictured by Jensen (plate 3) is suggestive of such a condition, and Jensen has always held this form of invasion to be the usual one. Debilitated animals would of course be most likely to succumb to such toxic assault. One may well conceive, however, that in a healthy animal in which some intestinal stasis occurred, or in one in which some highly concentrated suitable food were present for the rapidly multiplying anaerobes to grow on, an unusual multiplication of organisms might occur, and invasion might, in isolated cases, take place.

5. Some chemical agent in food (for example, poisonous plants) may break down the resistance that the animal tissues offer to the toxins and aggressins of the organisms.

6. The ingestion of earth may, as suggested by Gilruth, promote anaerobic alimentary canal invasion.

A final criticism of the research performed on this subject is in place. To my knowledge, a careful, thorough investigation either of the mixed flora or of pure strains of the anaerobes involved in the alimentary invasions of sheep has never been made. Elaborate technic for such study has not existed, but even such technic as has existed has not been applied. The study of the anaerobes is difficult and time consuming and the anaerobic field is enormous. The veterinary pathologist cannot alone hope to solve the problems involved, unless he become bacteriologist as well and spend many long months in the study of the organisms with which he experiments. Unless the investigator has time for such study, experimental research on this complicated problem will lead nowhere. It is a problem to be undertaken seriously by a laboratory having ample funds and many experimental animals and by a worker who is able to devote his full time

to the subject. If he have the services of a trained epidemiologist, so much the better. Fragmentary inoculation experiments with organisms of whose nature and relative distribution we know nothing lead us to confusion instead of to knowledge.

3. REINDEERPEST

A discussion of this disease must not be omitted.

Lundgren first reported it in detail in 1898. He brought with him from Lapland several tubes of pericardial and peritoneal and pleural fluid from a reindeer on which he made a necropsy examination. This material served for an elaborate and painstaking investigation by Bergman, who published an account of his work in 1901.

So far as these two publications show, there has been reported by an experienced pathologist only one necropsy examination on a reindeer supposedly dead of the spontaneous form of the disease, namely, Lundgren's case. Bacteriologic investigation is reported on material from this single case. Identification of this case with the other cases of the diseases known as reindeerpest rests entirely with the Lapps. The Lapps are a somewhat primitive people of exceedingly keen observation and scientific perception; especially, as Bergman says, may they be depended on to report with accuracy observations concerning reindeer. But I do not think that Lundgren and Bergman are justified in concluding that the pathogenic organism which was evidently the cause of the death of this reindeer is the one etiologic agent involved in the causation of reindeerpest or even in calling it the bacillus of reindeerpest.

Reindeerpest is a disease of the summer time and of the valleys. Reindeer are kept for most of the summer in the mountains on account of insects, and if they are brought down to the valleys they may die in large numbers of reindeerpest. When the herd is taken again to the uplands the disease disappears, when brought down again it reappears, only to die out in September. Epidemiologically the disease differs markedly from the diseases already discussed in only one respect, namely, that a large number of animals are attacked in a herd. There are three possibilities in its epidemiologic classification: that it is a contagious disease, or as Lundgren suggests, that it is an insect-borne disease, or that it is a disease introduced through abrasions or wounds, which diseases are probably governed in their ability to infect by several factors that may vary in different localities. Lundgren notes, for example, that during the summer when reindeerpest was most prevalent there was also a great deal of hoof disease among the animals, and he suggests that the pest originates as an infection of the diseased hoofs that partakes of the nature of a wound infection. The conditions in Lapland are different in many ways from those farther south. If it is true that the incidence of the disease ceases when the animals are driven into the mountains, the contagious nature of the disease is thrown into doubt, thus making it less likely that it is of the hemorrhagic septicemia type. Insects are numerous during the summer in the valleys of Lapland, and the reindeer is one of the animals most persecuted by them. The incidence of the disease in valleys and its occurrence in summer resembles the incidence and occurrence of blackleg. Valleys are highroads and are muddy; insects are there most abundant. The Lapps are an inquiring people. Bergman says that they often perform a necropsy examination on their dead reindeer to find out the cause of death, and they keep and use the skins and

let the bodies lie. The latter conditions favor anaerobic infection. As to the natural mode of infection of this disease, nothing is known. Young animals are the victims, even very young ones.

Bacterial Studies: Four of the five samples brought to Bergman by Lundgren were contaminated by mold. Bergman reproduced with them in various animals a disease in most ways similar to the disease in the reindeer on which Lundgren performed necropsy examination. From the organs of these animals and from the transudates of the original animal Bergman invariably grew an aerobe that was a facultative anaerobe. This aerobe grew in colonies on the surface of gelatin plates. Bergman describes it minutely. In culture it grew abundantly in the form of heavy rods 1.6x0.8 mikron, and reluctantly formed spores. It was a gas and acid former and grew on all mediums. It resembled several strains of sporulating aerobes that I have encountered. In the animal body the morphology of the organism, as shown by Lundgren's photographs and by Bergman's photographs, is most astoundingly like that of an organism of the vibrion septique group. Moreover, Bergman's bacillus forms long chains on the peritoneal surface and liver surface of animals. His account of the mode of spore formation of his bacillus is also characteristic of that of a vibrion septique type of organism.

Bergman's bacillus was highly pathogenic for reindeer, sheep, guinea-pigs and white mice, and it was also pathogenic for a calf, cats, brown rats, pigeons, and sparrows, and for frogs at 27 degrees. It was not pathogenic for rabbits, hogs, dogs, and chickens. I would suggest that there are not many species of organisms known that are so widely pathogenic. Bergman immunized sheep with sublethal doses of his organism. This animal was not immune to blackleg.

The pathogenicity of Bergman's material decreased rapidly in liquid cultures and not quite so rapidly in cultures on solid mediums. Pathogenicity could be recovered by animal inoculation.

We have here before us two possibilities: First, An aerobic organism exists (and we know of no other such aerobes) that is most strikingly similar (in morphology, incidence, pathogenicity, and in the lesions that it produces) to the organisms of the vibrion septique group, but which rapidly loses pathogenicity in aerobic cultures. Second, Bergman had to do with a symbiotic mixed infection of an organism of the vibrion septique group and an aerobic sporebearer, the latter able to render conditions suitable for the growth of the former on a plate. One hesitates to suggest the second contingency, for Bergman was evidently a careful worker and himself tried to demonstrate the absence of such a possibility; but it seems to me that such a possibility cannot be ignored. Bergman does not tell us of a careful examination of anaerobic colonies, or whether anaerobic cultures lost their virulence rapidly. He tells us of a dried gelatin plate with colonies, individual ones of which were nonpathogenic. "But if there were in the whole plate only one colony that contained

virulent reindeerpest bacilli, that would be sufficient to infect a reindeer." So he washed off the plate and inoculated the suspension into a reindeer and produced the disease.

I should suggest that if the losses from reindeerpest are considerable today, it would be worth while to study many cases of the disease, or at least several cases from various valleys. Investigation of the diseases of reindeer is, however, according to Lundgren, an extremely arduous pastime. Bergman's work is interesting and a step in the right path, but little can be done with material from one specimen. It must be borne in mind that anaerobic invasion may at times be a fatal one secondary to another type of infection.

4. THE ANAEROBIC INFECTIONS OF THE HORSE

I believe that earlier reports tend to diagnose horse infections as blackleg more frequently than do later ones.

Arloing, Cornevin, and Thomas (86) failed repeatedly to produce more than a local swelling in horses and asses by inoculating blackleg virus.

Hutyra and Marek (p. 34), in referring to malignant edema, say: "For natural infection of domestic animals solipeds are most susceptible."

Diedrichs reports investigation of material from the two horses that died of a Rauschbrand-like disease.

The material from the first horse produced what were apparently mixed infections fatal to guinea-pigs and pigeons. Cultures failed later to kill guinea-pigs. The culture was identified as Pseudorauschbrand in the Hygienic Institute of Berlin. The second strain remained proteolytic through several inoculations; but a guinea-pig, inoculated with a culture that had had several animal passages had little putrefactive odor and showed a colorless edema as thick as a finger; no gas is mentioned. The mixed (proteolytic) culture killed rabbits and was somewhat pathogenic for pigeons. It would seem that oedematiens-group infection is not improbable in this case. Infections of the vibrion septique group are probably never accompanied by proteolytic organisms through several passages. They do not produce edema as thick as a finger, and they produce gas. According to Diedrichs, the organism was not blackleg, and the pathogenicity tests apparently bear him out.

Von Hibler reports no cases of anaerobic infection in horses. He isolated B. oedematis maligni "in sinne von Koch" (Von Hibler X) from a mule.

Weinberg and Séguin report (b, p. 320) the case of a mule used for making antivenin. As a result of the venin inoculation a mixed infection of the head and neck developed. Edema was extensive, as was the tissue destruction, the latter being almost unbelievable in its extent. The infection was apparently due to B. histolyticus and B. oedematiens. The animal recovered after inoculations of mixed serum.

No samples of material from horses had come to this laboratory until Dr. Wood was so kind as to furnish me with four specimens, which were derived from horses that were being used for an immunity experiment. The pathogenic strain from one of the four horses was a typical vibrion septique: of interest is the fact that the animal, which had developed an extensive edema, recovered spontaneously from the infection.

The three other samples contained an interesting organism. Careful tests showed the three strains to be alike. The infected horses all succumbed, showing on section edema and gas formation. This organism (strains O 55, O 51, O 09) behaves much as do oedematiens strains. Inoculation of 0.5 c c of 24-hour meat medium culture of this bacillus into the thigh of a guinea-pig may kill the animal in 18 hours or less without any invasion of the body by the bacilli, or if little toxin be present the infection kills in 24 hours with septicemic invasion. A white gelatinous edema, that does not rapidly lose its gelatinous consistency on section, is found to surround the inoculated region. It is slightly blood stained. No gas formation takes place. A marked injection of the abdominal serous linings is the notable internal lesion.

On meat medium the organism produces a little gas, occasionally turning the meat pinkish, but producing so little acid that the pink color soon fades. The short, rather heavy bacilli are scarce in the medium, they stain palely as a rule, do not sporulate actively, and in 48 hours many individuals show the peculiar ghost-like appearance characteristic of oedematiens strains. Auto-agglutination takes place. The growth on milk is like that of B. oedematiens: reluctant and slow, very little acid being produced. On blood broth H_2S is produced, otherwise proteolysis is not patent. In colony formation, however, this organism is definitely to be distinguished from all the oedematiens-group strains in my collection.

Christiansen (a) found that normal horse serum protects guinea-pigs against infection with B. welchii.

I think that it can be stated that horses are subject to infection by organisms of the vibrion septique group and that they are subject to infection by organisms of the oedematiens group. I do not believe that any case of true blackleg infection in the horse has been established.

5. ANAEROBIC INFECTIONS OF THE HOG

It is apparent in the case of hog infections, as it was in the case of horse infections, that early diagnoses were more generally referred to blackleg and more recent diagnoses have been referred to the organisms of the vibrion septique group.

Arloing, Cornevin, and Thomas (p. 89) failed on repeated trials on all types of hogs to produce blackleg by inoculation. They state (p. 90) that hogs are susceptible to gas gangrene.

K. F. Meyer (a) reviews this subject. "The organism isolated from hogs by Marek, even though morphologically identical with B. chauvoei, had a high pathogenicity for rabbits; the publications of Marek do not contain any detailed account as to the biochemical actions of the observed organism. The same remarks apply to the publications of Born and Battistini in which, based purely on morphologic similarity of the observed organisms in the diseased muscles, the diagnosis of symptomatic anthrax in hogs was made." Meyer reviews the transmission experiments of von Ratz and holds that the lesions described by von Ratz so closely resembled those produced in hogs by the bacillus of Ghon and Sachs as identified by himself that he doubts, in the absence of cultural studies, whether von Ratz was really working with true symptomatic anthrax. He calls attention to the work of Glässner and of Wulff, who attempted to infect hogs with blackleg material and failed to produce more than a local swelling. Meyer says: "I cannot therefore agree with v. Ratz that hogs are susceptible to symptomatic anthrax."

Dr. Haslam has sent me a culture from one of several hogs that died of an anaerobic infection. The organism is a typical strain of the vibrion septique group.

Von Hibler reports a case of infection in a wild hog that was caused by B. novyi.

The lesions resembled those of Rauschbrand. Von Hibler was in possession of Novy's original strain of B. oedematis maligni II. This strain and others resembling it do not in my hands produce in the guinea-pig an infection remotely resembling blackleg. B. oedematiens Weinberg, strain Joly, does, however, produce a black hemorrhagic edema, as black as that produced by blackleg organisms. B. oedematiens and B. oedematis maligni II. are closely related and should logically be placed in the same group. It would be interesting to examine the strain isolated from the hog by von Hibler. One must remember that a hog and a guinea-pig might show different lesions in the same infection.

Von Hibler also isolated B. welchii (B. phlegmones emphysematoseae) from a hog that was thought to have died of pest.

I think it may be stated that hogs are susceptible to infection by organisms of the vibrion septique group, especially when they are weakened by other diseases, or when they are wounded. I do not believe that there is only one such invader that can be termed specific.

The question of invasion of hog tissue by organisms of the blackleg group is to my mind still an open one. These organisms are in general less pathogenic than those of the vibrion septique group. To prove anything in regard to this point a number of indubitable strains of the blackleg group of differing characters should be used. Probably exhaustive study would show that strains of the blackleg group are distinctly less pathogenic for hogs than strains of the vibrion septique group, and are incapable of infecting sound tissue of healthy animals, but that, were a wound severe enough, a blackleg strain would be capable of penetrating hog tissue, as was the Welch strain in von Hibler's case. The organisms of such wound infections should be looked on as relative in their pathogenicity; their action depends on so many factors that they cannot be considered in the light of our conceptions of ordinary infectious diseases.

Oedematiens group organisms are pathogenic for hogs (von Hibler).

B. welchii (B. phlegmones emphysematoseae, Fränkel) may be pathogenic for hogs. Von Hibler does not state how his hog may have contracted the infection.

6. ANAEROBIC INFECTIONS OF THE DOG

Evidently dogs and cats are more highly resistant to anaerobe infection than are herbivores, or we should hear more of such infections. It must also be borne in mind that carnivores are far more agile and skilful animals, and in the domesticated state are probably far less subject to wounding than are the herbivores.

7. AN EXPERIMENTAL INFECTION IN A CAMEL

An experimental infection in a camel is reported by Cross. He inoculated with blackleg virus a 7 year old camel and 2 yearling camels. All died of the infection.

8. ANAEROBIC INFECTIONS OF GAME

Ott and Ströse state that malignant edema is rare in game animals and that Rauschbrand has not been reported in game animals.

Budd, in 1863, quotes Hintermeyer who described in 1846 an outbreak of "quarter evil" in a herd of deer in a park in Germnay. There was an exudate from the nose, anus, and vagina of the animals. Just what the disease was we can probably not determine.

9. ANAEROBIC INFECTIONS OF RODENTS

Guinea-Pigs: I have observed two cases of spontaneous infection in the guinea-pig, which were caused by organisms of the vibrion septique group.

I believe that, in general, mice, rats, and rabbits are less susceptible to anaerobic invasion than are guinea-pigs, but this may be merely an impression. Cornevin found the guinea-pig most susceptible to experimental anaerobic infections.

10. WHALE SEPTICEMIA

In 1888 Nielsen (b) discovered and described a septicemia of whales that was due to wound infection. The natives of a region near Bergen caught whales by shooting them with infected arrows and then waited a day or two for the infection to develop before harpooning the animals. Nielsen described the causative organism of this infection and declared it to be similar to (but not identical with) the causative organism of blackleg. Recently M. Christiansen (b) has made a close study of an organism isolated by him from several samples of muscle from a single whale, which were taken in the late eighties. He places the organism in the same group as the bacillus of Ghon and Sachs, for which he proposes the name "Ghon-Sachs group," and he shows various characters differentiating his organism from B. chauvoei. Morphologically and pathogenically Christiansen's organism is distinctly to be placed in such a group, in spite of the fact that he insists that it is not flagellate, which character he believes to be an unimportant one. I have myself isolated an organism from whale muscle, which was kindly furnished me by Dr. C. O. Jensen. Dr. Christiansen states that my strain is identical with his strains; they all come from the same whale. I find that my strain is definitely referable to the vibrion septique group, but it is markedly distinct from any other strain in my possession. I should be much surprised if only one species were involved in all these wound infections of the whale. Very likely, however, one species was more pathogenic for whales than others and so became the predominant or more usual invader.

Obst found in 287 swelled cans of sardines an anaerobic organism "in pure culture." Assisted by W. G. Smillie she identified the organism as B. wallfischrauschbrand Nielsen. No animal experiments are recorded, and no standard anaerobe mediums were used in this study. The organism formed gas from protein and carbohydrate mediums; a foul odor was present in broth cultures. It sporulated heavily, forming "round" spores, giving the bacillus a "tennis racket formation." I can see no possible justification in thus identifying an anaerobe. It is counter to all chances that the anaerobic organisms in the 287 cans were in pure culture in the cans, and that the organisms in the 287 cans were the same. There is probably ample justification for identifying the bacillus of Christiansen with that of Nielsen. This organism is not proteolytic and forms oval spores. The aerobist should be warned that there are a thousand chances to one that a stray proteolytic anaerobe picked up from anywhere has not been described. Moreover, the possibility of any accurate identification of such an organism from a printed description is slight. The most we are today justified in doing when we wish to identify an anaerobe is to determine its group affinities. A careful and detailed description of a pure culture of the organism is then in order. Anaerobic literature is in no condition to be referred to for specific identifications of more than a scant handful of organisms; an authoritative systematic work on the subject does not exist.

11. NONMAMMALIAN ANAEROBIC INFECTIONS

Birds seem to vary in their susceptibility to anaerobic infections. Fowls are usually or invariably reported as immune. Pigeons are apparently subject to artificial infection with some strains of the organisms of the vibrion septique group but not to blackleg; they are highly susceptible to Welch bacillus infection. Sparrows may be killed by some vibrion septique strains.

Arloing, Cornevin, and Thomas (p. 91) produced infection by blackleg in frogs that had been kept at 22 C. in water. The organism of Bergman (see p. 438), which is not proved to be an anaerobe, infected frogs kept at 27 C. in air.

12. THE NOMENCLATURE OF ANAEROBIC DISEASES

It has probably been noted that the subject of the terminology of anaerobic infections is in an exceedingly chaotic condition. This is primarily because human and veterinary clinicians and pathologists have frequently attempted to diagnose the diseases seen by them according to the causative organism that they imagine is involved in a given case, instead of describing the disease as they find it and sending properly collected specimens to the bacteriologist for examination. Moreover, it is my experience that an opinion based on clinical and microscopic findings early in the examination of material from a case, is likely to prove mistaken or incomplete. Far too often has the bacteriologist, who found in a gas gangrene sample the ubiquitous B. welchii, contented himself with this finding and searched no farther.

Ghon and Sachs, who carefully reviewed the subject of nomenclature in 1903-04, came to a very simple conclusion, which, were the facts as they then appeared to me, could be accepted as a basis of terminology. Their idea was to term "malignant edema" the disease caused by the Bacillus oedematis maligni (under which name they identified Koch's, Pasteur's, and their own organism) and to call "malignant emphysema" the disease caused by Bacillus phlegmones emphysematoseae, Fraenkel. They state that this system has its shortcomings, but they do not wish to introduce more terms into the nomenclature.

Not to introduce new names when new conceptions arise would be hyperconservative; but it would seem also that the definition and application of old names needs revision. The whole field of anaerobic invasion must first be glanced over. We have a number of diseases to consider, which vary:

1. According to their location in the animal body: subcutis, muscle, pleural and peritoneal linings, glandular organs, alimentary canal.

2. According to their mode of inoculation: wound infections demonstrable as such, and infections whose point of entrance is obscure.

3. According to their distribution from the site of inoculation: through lymph or blood channels, through muscle or connective tissue spaces, or along lining surfaces.

4. According to the specific nature of the host, its age, and its condition.

5. According to the primary group affinities of the invaders: proteolytic, nonproteolytic, or mixed proteolytic-nonproteolytic.

If the infection is nonproteolytic, it varies:

6. According to the species of the invaders involved: (I have good reason for believing that wound infections, at least, are far more frequently and more highly polyspecific than they are usually thought to be), or according to the species of the one invader.

7. According to the relationship between the toxicity, the aggressive powers, and the reproductive powers of the particular strain or species of the principal invader, on which depend edema production, gas production, transudate formation, muscle invasion and hemolysis.

The varieties of specific invaders may be thought of as moderately numerous.

It is, according to the above desiderata, quite ridiculous longer to chain any specific invader to any pathologic term. Such an effort would be futile and would continually embarrass the pathologist. What we need is a set of purely pathologic diagnostic terms; descriptive terms we have in plenty. We may use old names for the pathologic processes encountered whenever they are descriptive of the lesion to which they are applied; but in so doing we must discard entirely the implication of the expression of an opinion as to the identity of the organism causing the lesion. The human and veterinary pathologists sorely need such a system as this whenever the material that passes their hands is to be examined by a bacteriologist. The pathologist cannot possibly be expected to determine the specific cause of an anaerobic invasion. He must have not only the descriptive terms that he possesses, but also diagnostic terms that do not in any way commit him to specific or even generic bacteriologic determination. The identity of such terms is for the pathologists to decide on. The bacteriologist is then free to make a diagnosis of the group or specific affinities of the invading organisms. I propose, however, that the terms malignant edema be given to the pathologists to abandon or to use as they see fit, and the term "bacillus of malignant edema" be discarded forever from bacteriologic terminology. The name B. oedematis maligni will also have to be abandoned unless someone, somewhere, can produce a culture whose history can be traced to Koch's laboratory, and which reproduces, on subcutaneous inoculation into laboratory animals, the lesions described in "Zur Aetiologie des Milzbrandes." No literary identifications of other strains with that of Koch's description can scientifically be accepted. Adamson's proposal to use the name B. oedematis maligni for the sporogenes type of organism has no systematic precedent, is illogical, and is likely to increase the existing confusion in our terminology.

The use of the term "gas bacillus" is likewise to be discouraged because it has become more or less definitely associated with B. welchii, which is no more a gas bacillus than are the vibrion septique organisms.

The anatomic diagnosis of "Rauschbrand" or "blackleg" should, in my opinion, for legal purposes, include infections by members of the blackleg group and of the vibrion septique group, or else some other term should be devised to include both types. There is no logic in separating the two diseases and recompensing the farmer for cattle dying of one and not of the other; moreover, different local veterinarians will probably never agree in their diagnoses of these diseases. The bacteriologist's diagnosis should depend on the organism or organisms found, and the two workers, pathologist and bacteriologist, can thus work in harmony.

I do not think that we are as yet in a position to take a stand as to the terminology of the infections of sheep. There should be a term (and bradsot and braxy probably fill the bill) that is descriptive of a rapidly fatal disease of the alimentary canal, which is associated with abundant multiplication of anaerobic organisms in the tissue. No specific or even generic meaning should be implied by the expression "anaerobic organisms." "Bradsot" or "braxy" may be taken to apply to such a disease in sheep or in goats or in hogs, as Zeissler has used it, or in other animals as well (see cattle, p. ??). When a thorough investigation of the obscure cases in sheep has been made it will be time to revise the nomenclature of such diseases.

GENERAL CONCLUSIONS

For more detailed conclusions see pp. 388, 389, 411, 416, 419, 420, 425 and 434.

Cattle are subject to spontaneous infection by organisms of the blackleg group and, somewhat less frequently, by organisms of the vibrion septique group, both of which types of infection are usually diagnosed as "blackleg."

Sheep are subject to spontaneous infection by organisms of the vibrion septique group and somewhat less frequently by organisms of the blackleg group. Both types of infection are probably diagnosed at times as braxy, as blackleg, and as malignant edema. It is possible that other diseases that are not of anaerobic origin are at times diagnosed as "bradsot" or "braxy."

The possibility exists that reindeerpest, as described by Lundgren and Bergman, is an anaerobic infection.

Horses are subject to infection by members of the vibrion septique group. Such infection may or may not follow a wound. It was formerly frequently diagnosed as blackleg. Horses are also subject to infection by organisms of the edematiens group. True blackleg group infection in the horse has probably never been demonstrated.

Hogs are subject to infection by members of the vibrion septique group. Such infection was formerly generally diagnosed as blackleg but has more recently been diagnosed as "malignant edema," "Ghon-Sachs bacillus infection," "specific gas phlegmon of hogs," or "Bradsot," depending on the location of the process. Hogs have probably never been shown to suffer from spontaneous blackleg infections.

The comparative rarity of oedematiens-group infections in animals, except perhaps in the horse, and the great rarity of serious invasion of animal tissue by B. welchii are to be noted.

In general many species of herbivorous mammals are subject to spontaneous anaerobic infection, both following and not following demonstrable wounds, and apparently the animals most susceptible are

ruminants, cattle and sheep, and possibly reindeer. Carnivores and man are subject to anaerobic infection only when wounded or when seriously debilitated by sickness.

All immunization work in connection with anaerobic diseases depends on a recognition of the group (see pp. 388, 389) affinities of the anaerobic organisms infecting the animals of the particular district in which immunization is proposed.

The factors concernng anaerobic infection that are of most interest, namely, the epidemiologic factors, are almost wholly unexplained, and their demonstration would prove a most valuable contribution to science and to agriculture.

I wish to make a plea for the world-wide study of these infections. They cost every agricultural country vast sums every year, and notable steps in the direction of immunization have shown that such immunization is feasible. To decide definitely the mode of infection in these diseases and the incidence of the group and specific entities involved would be of immense value to any country and to the world at large. The specificity of the toxins and of the aggressins of the different members in each group must be investigated. There are a hundred interesting immunologic and epidemiologic problems to be suggested. The proper investigation of the subject can be done only in a laboratory that is well equipped to handle anaerobes and that has the funds to employ a trained epidemiologist who knows something of veterinary pathology and can spend all his time in the field.

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